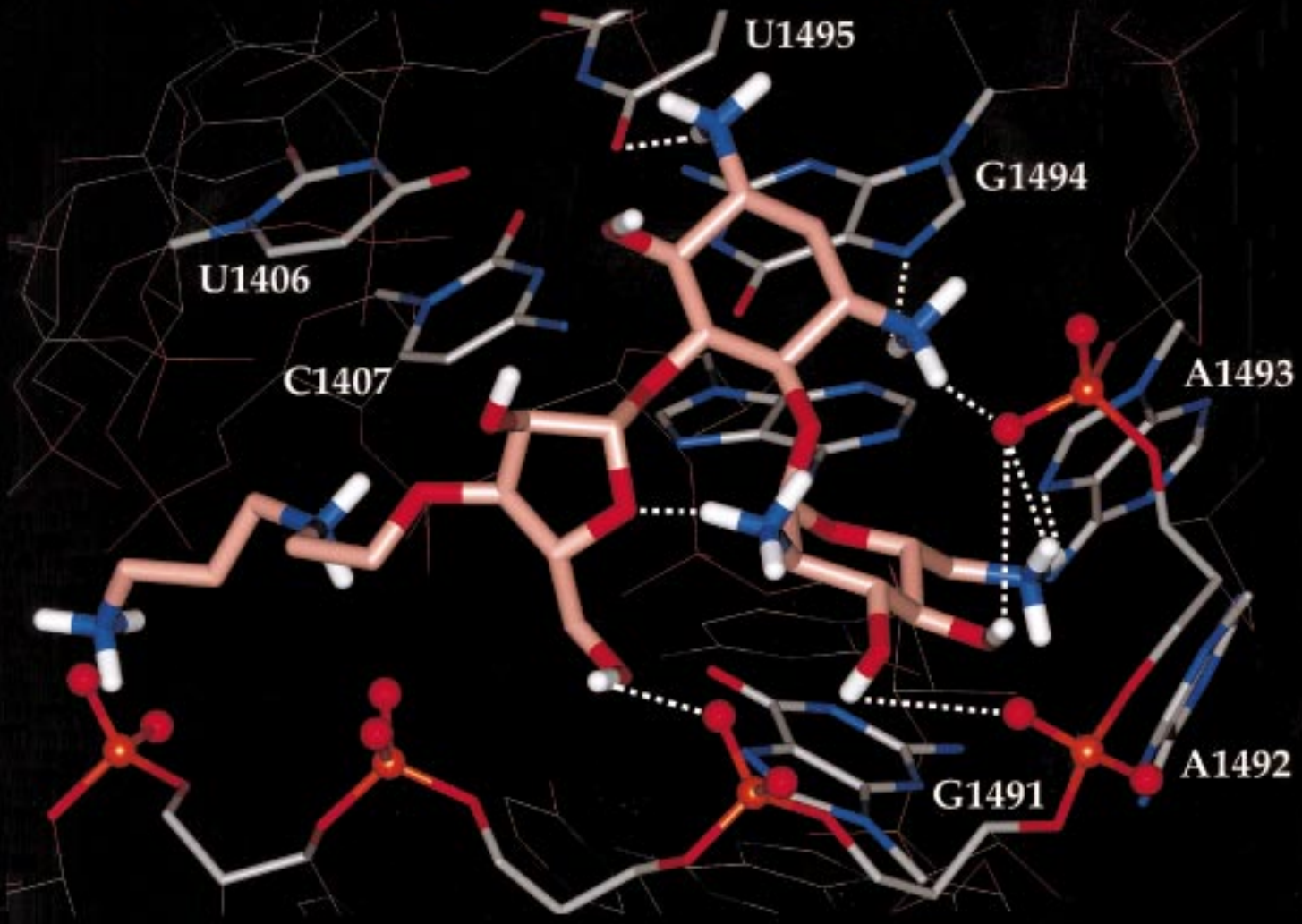
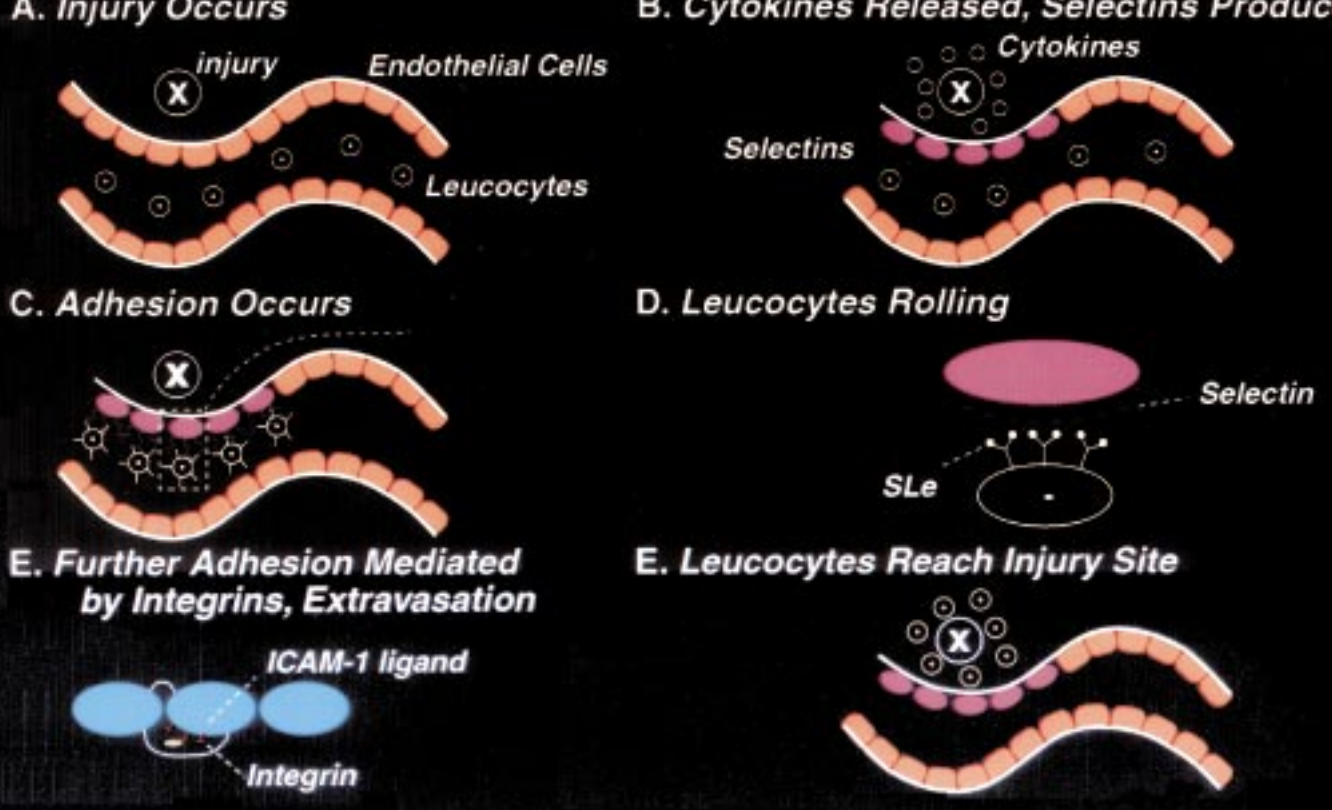
A Synthetic Aminoglycoside Mimetic Targeting Bacterial rRNA



Inflammatory Reaction Involves Selectin-Carbohydrate Interaction



Carbohydrate Mimetics: A New Strategy for Tackling the Problem of Carbohydrate-Mediated Biological Recognition

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Dedicated to Professor George M. Whitesides

Of the three major classes of biomolecules—proteins, nucleic acids, and carbohydrates—it is the carbohydrates that are the least exploited. Despite the important roles that saccharides play in numerous biological recognition events (e.g. bacterial and viral infection, cancer metastasis, and inflammatory reactions) the molecular details of these recognition processes are generally not well understood, and consequently the pace of development of carbohydrate-based therapeutics has been relatively slow. This slow pace of development is further hindered by the lack of practical synthetic and analytical methods available for carbohydrate research and by the problems associated with undesirable physical chemical properties of saccharides as drug candidates. Recent advances in the field, however, have demonstrated that these problems can be circumvented with the use of carbohydrate mimetics, that is, small molecules that contain the essential functional groups (often with additional hydrophobic or charged groups) to resemble the active conformation of the parent structure.

Keywords: carbohydrate mimetics · carbohydrates · sialic acids

1. Introduction

Carbohydrates are ubiquitous and important biomolecules. Besides their role in energy storage, they form much of the structural framework of cells and tissues. As part of glycoproteins, glycolipids, and other conjugates, they are key elements in a variety of processes such as signaling, cell–cell communication, and molecular and cellular targeting.[1–3] For example, lectins (sugar-binding proteins) displayed on the surface of a cell allow it to respond to a variety of external stimuli such as the local concentration of nutrients (chemotaxis). The lectins also allow them to bind and localize cells displaying appropriate saccharides, a process typified by an early step of inflammatory response: E- and P-selectins, mammalian lectins displayed on the endothelial cell surface following cytokine stimulation, bind sialyl Lewis^X (sLe^X; NeuAcα2,3Galβ1,4(Fucα1,3)GlcNAc) and related oligosaccharides displayed on circulating leukocytes, leading to the attachment and eventual migration of the leukocyte into the surrounding tissue. A third function of lectins is to allow cells to select and take up glycosylated molecules or microorganisms. Pathogenic microorganisms are frequently ingested by macrophages in a lectin-mediated fashion. This has been observed, for example, in the uptake of the fungus *Pneumocystis carinii* by the macrophage mannose receptor.[4] Glycoproteins may be taken up in a similar fashion. Certain pituitary hormones (follicle-stimulating hormone, FSH; luteinizing hormone, LH) display GalNAc-4-sulfate, which is responsible for the uptake of these hormones by the Kupffer cells of the liver.[5]

Carbohydrate recognition events are involved in the progression of a number of diseases. The binding of many pathogens and biological toxins to the host cell surface is carbohydrate-mediated. *Helicobacter pylori*, a pathogen associated with gastritis and peptic ulcers and implicated in gastric carcinoma, has been reported to bind a wide variety of saccharides on the surface of the host cell, including Lewis b,[6] 3′-sialyllactose,[7] and others.[8] Such carbohydrate binding is a very common theme in bacterial colonization of tissues.[2] Viruses and toxins also frequently use oligosaccharide receptors; the influenza virus, for example, binds to sialic acid through its coat protein, haemagglutinin, and this event is prerequisite to viral entry into the host cell.[9] The potent plant toxins abrin and ricin bind to D-galactosides,[10] while bacterial AB_5 toxins, heterohexameric compounds in which the five B subunits are responsible for targeting, bind typically to gangliosides. Two examples are the cholera and shiga toxins

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which bind to G_{M1} and G_{b3}, respectively.[11] Tumor metastasis is proposed to occur in some cases by the same targeting pathway that leukocytes use: binding to endothelial cells through the sLe^a or sLe^X-selectin interactions, ultimately followed by extravasation.[12]

Although carbohydrate recognition events are frequently implicated in the progression of a disease, they can sometimes be part of the cure. The bacteriocidal aminoglycoside neomycin and related compounds are recognized by the bacterial ribosome. Binding to the ribosome causes translational inhibition and, at high concentrations, miscoding.[13] Lectin-mediated uptake has been put to good use in the treatment of Gaucher's disease, a genetic deficiency of the enzyme β-glucocerebrosidase. Treatment requires the administration of the enzyme, but it must be internalized into lysosomes, so glycosidases are used to trim the N-linked saccharide of the purified enzyme down to the pentamannose core; this allows its uptake by a mannose-binding lectin.[14]

The intervention or, in some cases, the mimicking of protein–saccharide or nucleic acid–saccharide interactions provides a potential target for therapeutic agents. Unfortunately, saccharides often do not make good therapeutic agents for a variety of reasons. Many natural saccharides are rapidly degraded by digestive, plasma, and cellular glycosidases, and frequently bind to their targets with low affinities, though polyvalency can be used to improve the low-affinity carbohydrate–receptor interactions on cell surfaces.[15] The K_d values are most often in the millimolar range, although there are exceptions. The arabinose-binding protein, for example, has been reported to bind arabinose with a K_d value of 0.098 μM;[16] the *Griffonia simplicifolia* lectin GS-IV binds the Lewis b antigen with a K_d value of 24 μM;[17] cholera toxin binds the ganglioside G_{M1} pentasaccharide with an intrinsic binding constant—that is, the binding of a single ligand by a single subunit within the pentamer—of 1 μM;[18] and the anti-*Salmonella* O-antigen monoclonal antibody Se155-4 binds the trisaccharide Abeα1,3(Galβ1,2)ManαOMe with a K_d value of 15 μM.[19] In addition, polysaccharides are usually difficult to synthesize by conventional organic chemical techniques, though the growing availability of glycosidases and glycosyltransferases and the introduction of cofactor recycling schemes has made an enzymatic approach to synthesis more feasible in recent years.[20, 21] New synthetic methods have also decreased the difficulty of chemical synthesis.[22–27] For these reasons, it is frequently desirable to design compounds that are mimics of carbohydrates associated with important signaling and recognition events, but have improved properties with regard to stability, specificity, affinity, and synthetic availability.

The term "carbohydrate mimetic" is frequently used to refer to any carbohydrate derivative or other compound that has multiple hydroxy groups and thus looks somewhat like a sugar or saccharide; we prefer to reserve the term for compounds that have been demonstrated to truly mimic the structural and functional aspects of a known target. These will be the focus of this review.[28]

Carbohydrate mimetics have a number of advantages over their parent structures as therapeutic agents. They can be designed such that they 1) are more stable toward endogenous degradative enzymes, 2) have improved bioavailability and reduced clearance rates, and 3) have a higher affinity and selectivity for their cognate receptors by taking advantage of interactions that the natural saccharide does not. By constructing polymers or oligomers with multiple copies of the mimetic to allow for polyvalent interactions, the affinity can be increased further.[15] In the case of inhibitors of glycosyl-

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transferases, glycosidases, and carbohydrate-modifying enzymes (e.g. sulfotransferase), mimetics can be designed that imitate the transition states of these reactions, rather than the ground states, and thus will inhibit the enzymes better than simple substrate analogues.

2. Structural Features of Carbohydrate – Protein Interactions

To make a good mimetic, it is important to be aware of the interactions between carbohydrates and their targets, which are usually proteins. A number of interactions are used to contribute to the negative free energy of binding.[29–31]

2.1. Hydrogen Bonding

Hydrogen bonds are, predictably, an important interaction. Hydrogen bonds between the carbonyl and NH groups of the protein backbone and the sugar hydroxy groups are very frequently observed in the available crystal structures of sugar-binding proteins. The heavy representation of such contacts is probably due both to the large number of amide bonds available and to the constrained nature of the peptide backbone: Since the backbone has fewer degrees of freedom than the side chains, the entropic loss during formation of the hydrogen bond due to the restriction of the positions of the hydrogen-bond donor and acceptor in space will be reduced if one or the other is already fixed. In contrast, the hydroxylated amino acids serine and threonine are used much less often, presumably due to the unfavorable entropy of fixing two moderately flexible—at least as compared to the peptide backbone—groups upon binding.[29] "Bidentate" hydrogen-bonding side chains such as those in aspartic acid, asparagine, glutamate, glutamine, and arginine are also commonly found. The two "arms" of such side chains can form hydrogen bonds to vicinal hydroxy groups. This is observed, for example, in the crystal structure of the arabinose-binding protein with bound galactose,[32] where the carbonyl group and amide nitrogen atom of the asparagine 232 side chain make hydrogen bonds with the 3- and 4-hydroxy groups of galactose (Figure 1). In the same structure, arginine 151 hydrogen bonds to both the 6-OH group and the ring oxygen atom of galactose. Many of the hydrogen bonds of sugar-binding proteins are water-mediated; although there is a tendency to consider water-mediated hydrogen bonds to be weak and nonspecific, this is really not accurate. In many of these cases the water involved is observed in the crystal structures of both the ligated and unoccupied binding sites, and can in essence be considered an extension of the protein.[16] For example, in the arabinose-binding protein (containing galactose), two water molecules are observed to hydrogen bond to three protein side chains each; the fourth hydrogen-bonding site is occupied by either the 2- or 6-OH group of the bound galactose. These water molecules are also observed in the crystal structures complexed with 2- or 6-deoxygalactose.[32]

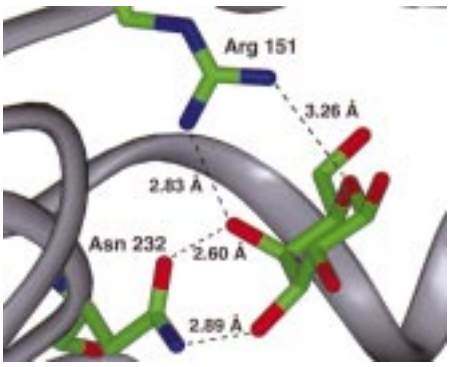


Figure 1. Schematic representation of the hydrogen bonds between β-D-galactose and two side chains of the arabinose-binding protein, showing the "bidentate" nature of the hydrogen-bonding pattern. The carbonyl oxygen atom and amide nitrogen atom of the asparagine 232 side chain form hydrogen bonds to vicinal hydroxy groups (3- and 4-OH) of the bound galactose. (Crystal structure 5abp.pdb by Quiocho and colleagues[32] retrieved from the Brookhaven Protein Databank and viewed with InsightII, MSI, San Diego, CA; red: oxygen, blue: nitrogen, green: carbon.)

2.2. Association with Metals

The C-type lectins and related proteins are calcium-dependent saccharide-binding proteins. The reason for the absolute dependence of sugar binding upon the metal is clear from the crystal structures: Vicinal hydroxy groups on the sugars coordinate with the calcium, as in mannose-binding protein A,[33] where the 3- and 4-hydroxy groups of mannose make contacts to calcium (Figure 2). A similar coordination is

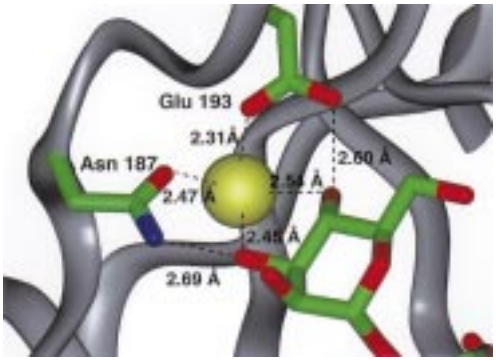


Figure 2. Coordination of vicinal hydroxy groups (3- and 4-OH) of mannose by calcium (yellow sphere) in the mannose-binding protein A. (Crystal structure 2msb.pdb by Weis and co-workers;[33] red: oxygen, blue: nitrogen, green: carbon.)

believed to occur in the binding of sialyl Lewis^x (NeuAcα2,3-Galβ1,4(Fucα1,3)GlcNAc) to E-selectin; vicinal hydroxy groups of fucose are thought to coordinate to the calcium at the binding site.[34, 35] The X-ray crystal structure of the enzyme xylose (glucose) isomerase containing (linear) glucose shows that the 2- and 4-OH groups and the carbonyl moiety of the bound glucose are coordinated to not one but two magnesium atoms which are required for catalytic activity.[36]

2.3. Hydrophobic Packing

Although sugars are frequently considered to be polar molecules, it has been observed for many years that they have substantial hydrophobic character associated with the faces. This is underscored, for example, by the ability of cyclodextrin to solubilize hydrophobic compounds and by the ability of linear dextrin (poly(Glcα1,4Glc)) to increase the water solubility of strongly hydrophobic compounds such as steroids.[37] Packing of a hydrophobic ring against sugar is observed in most saccharide-binding proteins,[38] and an excellent example of this can be observed in the crystal structure of the maltose-binding protein containing maltose.[39] Both glucose molecules of the bound disaccharide are sandwiched between aromatic rings (Figure 3). The faces of

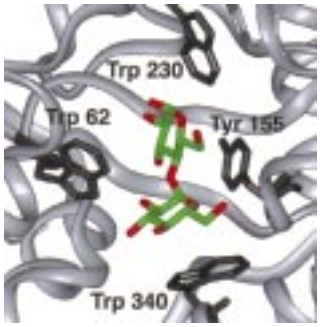


Figure 3. Interaction of aromatic side chains of the maltose-binding protein with the faces of the two glucose units of maltose. (Crystal structure 1anf.pdb by Quiocho and co-workers;[39] red: oxygen, green: carbon.)

the sugar and the aromatic ring are not always perfectly aligned; in many sugar-binding proteins, the plane of the aromatic group is canted somewhat with respect to the face of the sugar.[29] Some sugars have faces that are more hydrophobic than others. The faces are assigned a letter A or B, where the A face is the side on which the atoms progress from lower to higher number in a clockwise fashion.[40] In galactose, the 4-OH group points up on the A face, and the B face is correspondingly more hydrophobic for D-galactose than for glucose or mannose. This is perhaps why the B face of galactose nearly always has an aromatic ring sandwiched against it.[29]

2.4. Ionic Interactions

Ionic interactions are also observed in the complexation of proteins or nucleic acids with charged or derivatized sugars such as sialic acids, aminosugars, and phosphorylated or sulfated sugars. For example, sugar carboxylates often associate with arginine, as observed in the structure of influenza neuraminidase containing sialic acid (Figure 4);[41] likewise, much of the binding affinity of the aminoglycoside antibiotics for RNA is due to interactions of the 1,3-hydroxyamine motif of antibiotics with the phosphodiester backbone (Figure 5) as well as the Hoogsteen face of guanine.[42, 43]

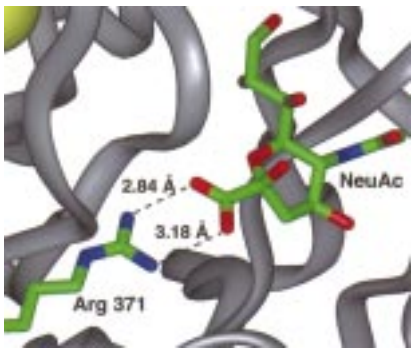


Figure 4. Interaction of the guanidino group of influenza neuraminidase Arg371 with the carboxylate moiety of sialic acid (NeuAc). (Crystal structure 2bat.pdb by Varghese and colleagues;[41] red: oxygen, blue: nitrogen, green: carbon.)

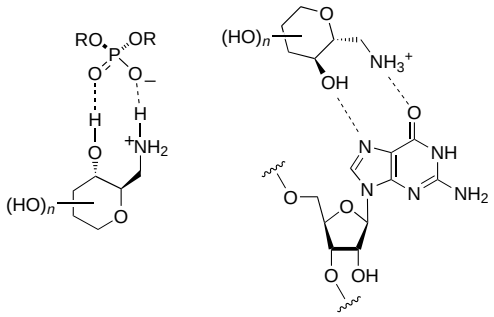


Figure 5. Interaction of 1,3-hydroxyamines with the phosphodiester backbone of nucleic acids and with the Hoogsteen face of guanine.

3. Strategy for the Design of Carbohydrate Mimetics

Understanding how carbohydrates interact with their protein receptors provides useful information for the design of carbohydrate mimetics. The weak affinity in sugar–protein interactions is attributed to a few causes: 1) There is a lack of hydrophobic groups in sugars which are often dominant in high-affinity receptor–ligand interactions; 2) the energy associated with multiply hydrogen bonded interactions in sugar–protein recognitions is significantly diminished by competition from bulk solvent; 3) the flexible nature of many hydrogen-bonding groups results in a significant entropic penalty when they become constrained in space upon binding. It also appears that not all functional groups of a carbohydrate ligand are essential for interaction with the receptor.

With this information in hand, one may choose a couple of strategies (Figure 6) for the design of organic molecules that mimic the active conformation and function of a carbohydrate ligand. In the first approach, one may remove unnecessary functional groups, but keep the original glycosidic linkages in order to retain the conformation (Figure 6, middle). This approach will reduce the polarity of the sugars, and may increase the affinity of the binding by improving hydrophobic interactions and reducing the penalty for desolvating polar functional groups on the sugar. The synthesis of deoxysugars is not trivial, however. Some deviations from this strategy have also been used in which functional groups are added or

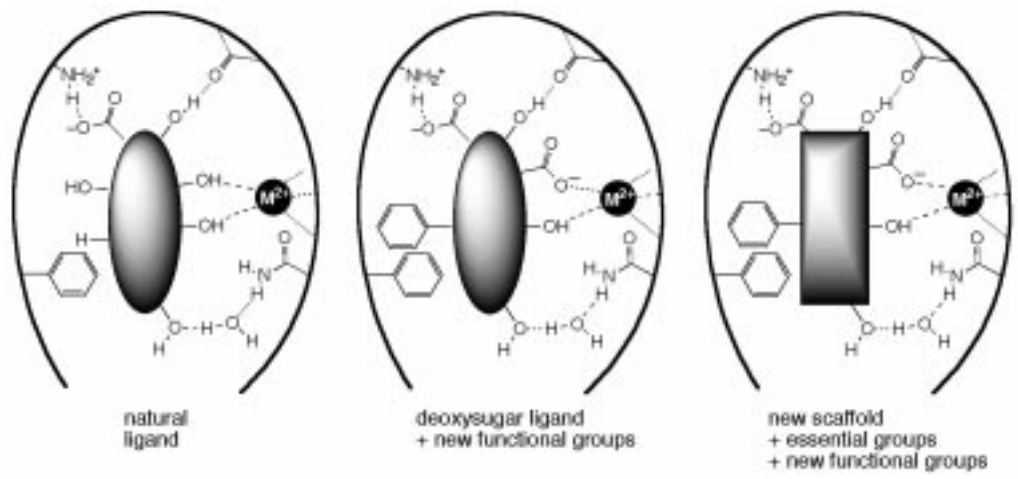


Figure 6. Schematic illustration of two general strategies for designing carbohydrate mimetics. The diagram on the left shows the interactions between a natural saccharide and a protein receptor. In the diagram in the middle a mimic is used in which the glycoside backbone is retained, but unnecessary hydroxyl groups are removed and additional functional groups added to strengthen the interaction. In the diagram on the right, the glycosidic scaffold is abandoned.

changed to further improve the affinity and/or stability. The incorporation of a hydrophobic group has the potential to substantially increase the binding affinity if there is a complementary hydrophobic site in the receptor. This is a potentially powerful technique. A charged group can add favorable ionic interactions if a complementary group exists in the binding site. In cases where the saccharide coordinates a metal, it may be possible to improve the affinity by replacing the coordinated hydroxy groups with a better metal ligand.

The stability may be improved by changing the O-linked to a C-linked (or S-linked) saccharide, or to O-linked carbocycles. Though the *exo*-anomeric and steric effects (Figure 7) are the main factors that govern the glycosidic torsion angles, the steric effect of C-linked saccharides has been found to play a major role in maintaining the conformation of C-linked saccharides in the *gauche* form, similar to the conformation in the corresponding O-linked sugars.[44] The C-linked glycosides are therefore often used to mimic the parent O-linked

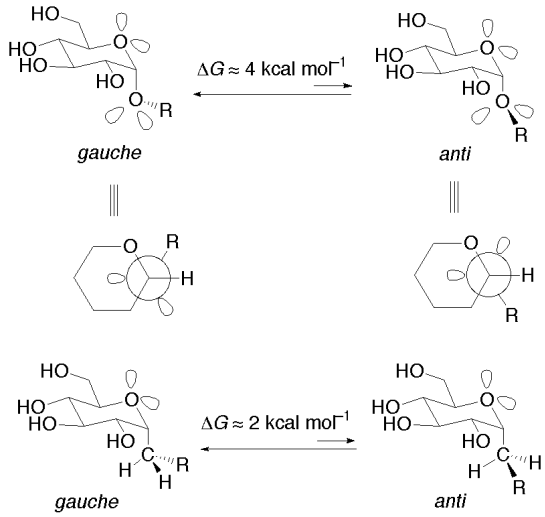


Figure 7. The *exo*-anomeric and steric effects on the conformation of a glycoside, and energetic consequence of changing an O-glycoside to a C-glycoside.[17]

structures as they are more stable toward acid- and glycosidase-catalyzed cleavage.

A second approach toward the construction of carbohydrate mimetics is to abandon the glycosidic scaffold entirely (Figure 6, right); instead, a new, non-carbohydrate framework is built and the required functional groups are attached so as to have the same orientation in space as they do in the parent structure. In addition, new hydrophobic or charged groups may be incorporated to further enhance the affinity.

A wide variety of molecules have been used to mimic carbohydrates and their derivatives. Some of those found in the natural world are shown in Figures 8–14, along with the structure that is mimicked. Tunicamycin (Figure 8) has long

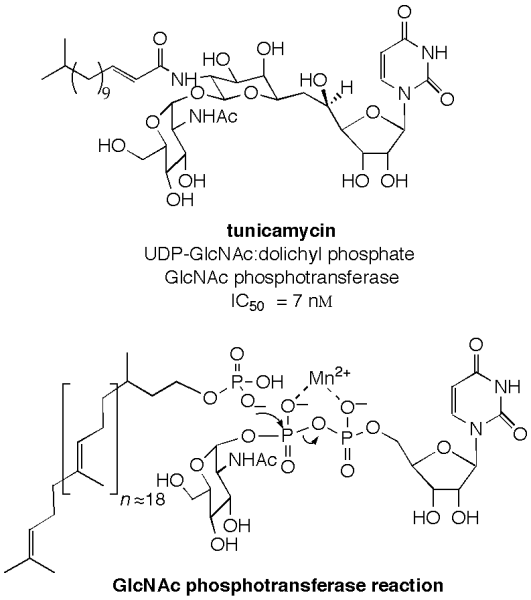


Figure 8. The GlcNAc phosphotransferase inhibitor tunicamycin[156] and the enzymatic reaction it mimics.

been known to be a potent inhibitor of UDP:GlcNAc:dolichyl phosphate *N*-acetylglucosaminyltransferase, the enzyme catalyzing the first committed step in the synthesis of the ultimate saccharide donor for the N-glycosylation of proteins.[1] A novel feature of this inhibitor is its mimicry of the pyrophosphate of the donor with galactosamine, which is C-linked to ribose. Moenomycin A (marketed as Flavomycin by Hoechst; Figure 9)[45] is an inhibitor of the bacterial transglycosylase responsible for the stepwise condensation of disaccharides to form the polysaccharide framework of the

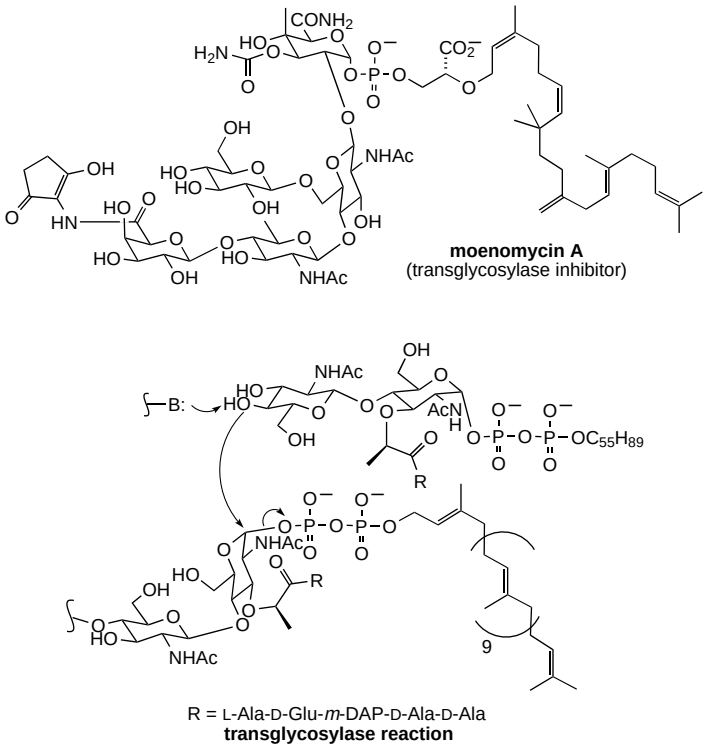


Figure 9. The transglycosylase inhibitor moenomycin A (structural revision in 1990[45]) and the transglycosylase reaction.

peptidoglycan cell wall. Studies have shown that much of the molecule can be removed with retention of activity,[46, 47] and possible transglycosylase inhibition has been recently demonstrated with a very different molecule derived from the disaccharide of vancomycin.[48] Several types of natural glycosidase inhibitors are shown in Figures 10 – 13, along with the putative transition states of the reactions inhibited.

Chemical synthesis has offered even more potential carbohydrate mimetics, particularly in the areas of nucleic acid

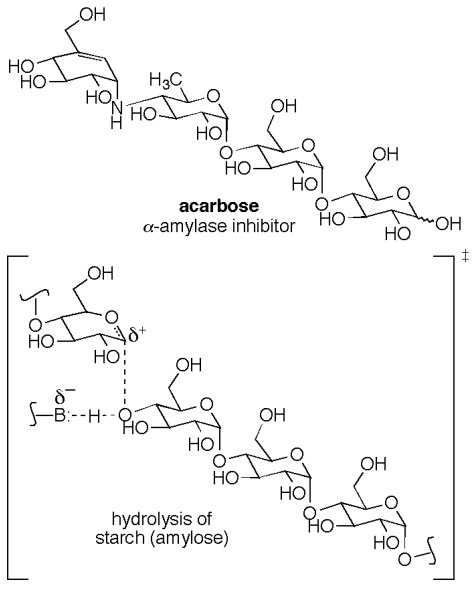


Figure 10. The α-amylase inhibitor acarbose and the amylase-catalyzed hydrolysis reaction.

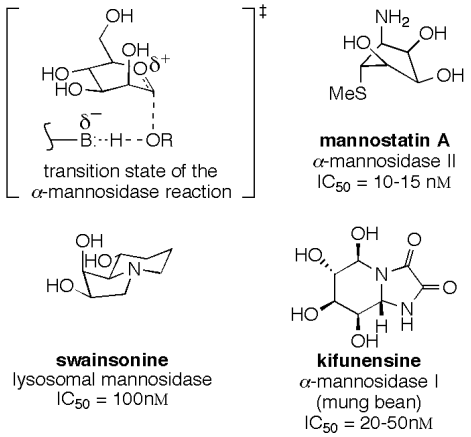


Figure 11. The mannosidase inhibitors mannostatin A,[157] swainsonine,[158] and kifunensine,[159] along with the putative transition state of the mannosidase-catalyzed reaction.

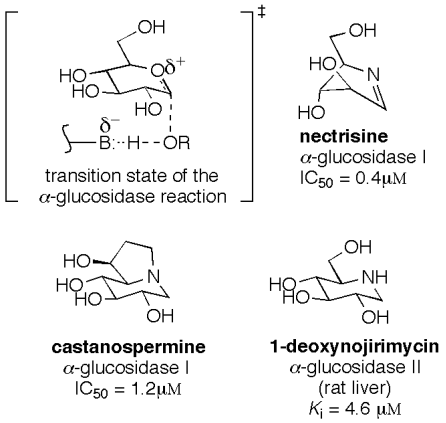


Figure 12. The α-glucosidase inhibitors nectrisine,[160] castanospermine,[160] and 1-deoxynojirimycin,[161] along with the putative transition state of the α-glucosidase-catalyzed reaction.

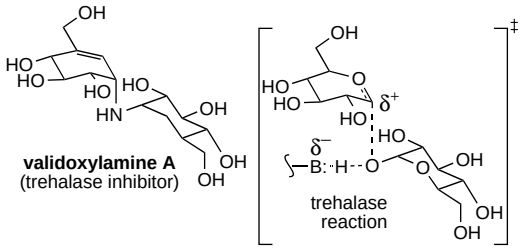


Figure 13. The trehalase inhibitor validoxamine A, along with the putative transition state of the trehalase-catalyzed reaction.

mimetics (a large field which has recently been reviewed by Verma and Eckstein[49] and will not be covered here) and glycosidase and glycosyltransferase inhibitors. A small sampling of the types of scaffolds used to mimic pyranose rings (or their hydrolytic transition states) is shown in Figure 14. More can be found in reviews such as those by Vasella et al.,[50] Tatsuta,[51] Legler,[52] and Heightman and Vasella;[53] the last focuses on recent attempts to discern detailed mechanistic information about configuration-retaining glycosidases through the design of transition state analogues. In the case of glycosyltransferase inhibitors, hydrolytically stable pyrophosphate analogues are also typically desirable. Much of the

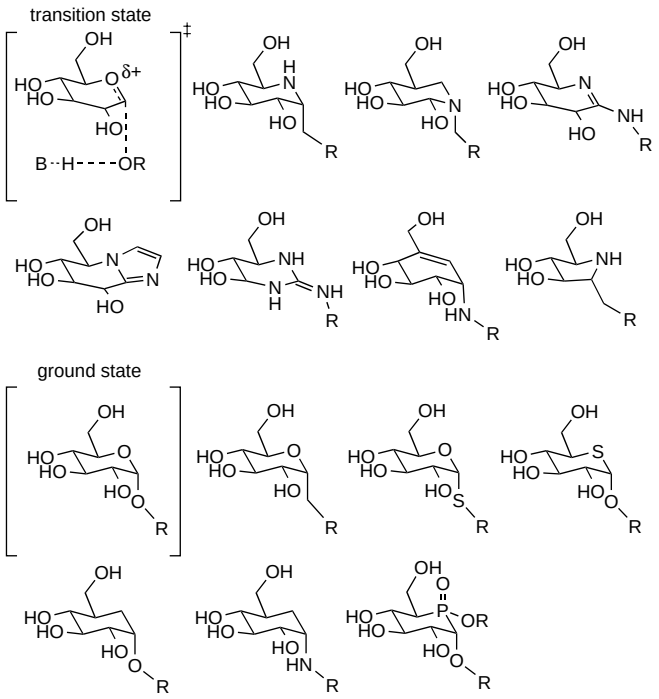


Figure 14. Some simple mimics of the ground state of a glycoside (recognized by a receptor) and of the transition state for glycoside hydrolysis (recognized by an enzyme).

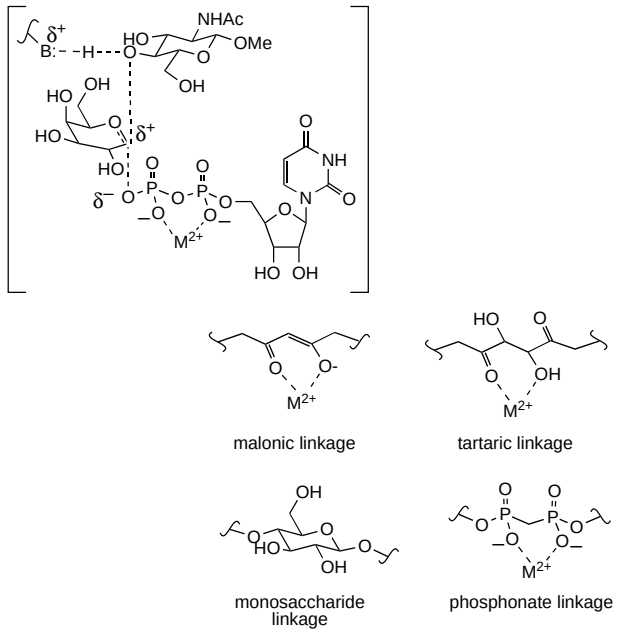


Figure 15. The putative transition state of galactosyltransferase showing the complexation of the pyrophosphate by an active-site divalent metal (particularly Mn^{2+} or Mg^{2+}), along with some pyrophosphate mimics that have been used.[54]

binding energy of the sugar donor comes from the binding of the leaving group nucleotide, as evidenced by the fact that the released nucleotide is inevitably a strong competitive inhibitor of glycosyltransferases. Analogues of just the sugar portion of the transition state have rarely been observed to be good inhibitors of these transferases. Better inhibitors have incorporated either donor nucleotide mimics, acceptor mimics, or both. Figures 8–10 show a number of natural compounds which mimic pyrophosphate with sugars, carboxylates, and carbonyls. A sampling of some synthetic pyrophosphate mimics is shown in Figure 15. Of these, the phosphonate and monosaccharide linkages have shown the most promise.[54]

4. Saccharide Synthesis and Processing Enzymes

Oligosaccharides such as those found on cell surfaces are assembled through the action of glycosyltransferases and glycosidases. Saccharides may be attached to protein through a number of different linkages, but most commonly the links are to asparagine (N-linked) or threonine/serine (O-linked). Although O-glycosylation proceeds by the relatively straightforward addition of a single sugar to a hydroxylated side chain and elaboration with glycosyltransferases, N-linked glycosylation is more complex. It proceeds through a pathway in which a large oligosaccharide is built on a specialized phospholipid, dolichyl pyrophosphate. This is transferred to a newly synthesized protein in the endoplasmic reticulum, and glycosidases (glucosidases, mannosidases) may nibble the saccharide back to a much smaller core before glycosyltransferases elaborate it to the mature N-glycan.[1]

Further elaboration of oligosaccharides by acetylation, sulfation, and phosphorylation may also occur to form the mature saccharide recognition motif.[1] Glycosidases and glycosyltransferases are important targets for intervention for a number of reasons. The progression of many diseases is dependent on the action of these biocatalysts. For example, changes in glycosylation are a well-documented characteristic of cancer cells,[55] and are frequently associated with their metastatic potential. In particular, cancer cells are more heavily sialylated than their normal counterparts. Their N-glycans are more heavily branched,[56, 57] as a general rule, whereas the O-linked glycans tend to be truncated. The Tn-(GalNAc-Ser/Thr) and T-antigens (Galβ1,4GalNAc-Ser/Thr) and their sialylated versions are also much more prevalent on tumor cells.[58]

Pretreatment of melanoma cells with tunicamycin (Figure 8), a compound that inhibits the first enzymatic step in protein N-glycosylation (UDP-GlcNAc:dolichyl phosphate GlcNAc phosphotransferase), reduces their binding to epithelial cells in culture.[59] Tunicamycin is, however, much too toxic to be used clinically; many inhibitors of enzymes that catalyze very early steps in the glycan processing pathway cause severe neurological toxicity. More selective inhibitors are needed which inhibit only one or a few glycosylation enzymes. The activity of glycosidases is also implicated in metastasis. Glycosidases within the endoplasmic reticulum and Golgi apparatus are required to trim immature N-glycans prior to their elaboration into more complex structures,[1] while secreted glycosidases degrade the extracellular matrix and allow tumor cells to "escape" into the circulation. The enzymes involved in the acetylation, phosphorylation, and sulfation of carbohydrates are less studied, but may also become important targets for intervention.

Various mechanistic studies have indicated that these glycosidase and glycosyltransferase reactions proceed through an S_N1-type mechanism,[53, 60–62] in which the leaving group mostly leaves before the incoming nucleophile (water in the case of glycosidases, or a sugar hydroxy group or other nucleophile in the case of the glycosyltransferases) attacks. The transition state is believed to have a distorted ring in a half-chair conformation, with substantial positive charge on the anomeric carbon atom that is somewhat delocalized to the ring oxygen atom (Figure 16). This view is supported by many

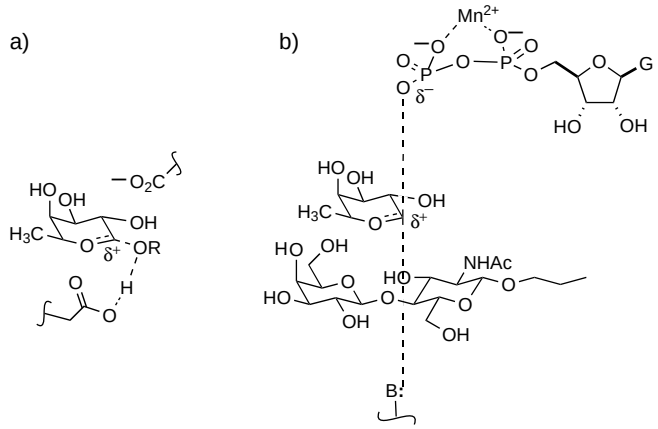


Figure 16. Transition states of a) fucosidase and b) fucosyltransferase reactions.[63] G = guanine.

lines of evidence, including studies of kinetic isotope effects[63–67] and by the strong inhibitory power of compounds that mimic[53] or destabilize[68, 69] this flattened ring oxocarbenium intermediate. A large variety of molecules have been synthesized which mimic the proposed transition state of various glycoside-hydrolyzing enzymes. The distorted ring is mimicked with rings of altered size by incorporating one or more sp^2 centers in the ring (e.g. unsaturated carbacycles, amidino, or guanidino sugars) or by perturbing the conformation with, for example, bicyclic systems or bridged rings. The positive charge, likewise, is typically mimicked by the addition of a basic group such as an amine, guanidine, or amidine. Attaching these compounds to a leaving group (a sugar) or its mimic will provide more selective inhibitors of glycosidases.[68, 70] On the other hand, attaching them to nucleotides or nucleotide analogues frequently provides inhibitors of the cognate glycosyltransferases as well. Many of the resulting compounds, some of which are illustrated in Figure 17, are powerful inhibitors of glycosidases. It is clear that inhibition can be very sensitive to the position of the sp^2-hybridized atoms within the ring (e.g. compounds g versus h in Figure 17) and to the position of the positive charge (e.g. compound i versus j in Figure 17). Some of the nitrogen-containing heterocycles described above, particularly the five-membered iminocyclitols, have also been used as haptens to elicit catalytic antibodies that catalyze glycoside cleavage.[71] An alternate approach in the inhibition of configuration-retaining glycosidases, which proceed by a double-inversion mechanism with a covalent enzyme–substrate intermediate,[72, 73] is to use a substrate that forms a stable covalent intermediate, such as a 2- or 5-fluoroglycoside.[68, 69] By using a fluoroglycoside with a good leaving group (either an activated glycoside such as the 2,4-dinitrophenolate, or, more recently, simply the enzyme's preferred aglycon[68]), the enzyme can catalyze the initial step to release the leaving group and form the covalent intermediate. Subsequent hydrolysis is difficult, however, due to destabilization of the oxocarbenium transition state by the fluorine.

5. Mimics of Mono- and Oligosaccharide Ligands for Receptors

Simple sugar mimics can be extended to create oligosaccharide mimics, but the mode of attachment must be designed with care. Saccharides bind to their receptors with a well-defined conformation, which may well be different from that in solution, as in the case of the sialyl $Lewis^X$ binding to E-selectin (discussed in Section 5.1). There are several instances in the literature where attachment of sugars with nonglycosidic bonds abrogates binding to the receptor or an antibody. For example, the "carbopeptoids" (amide-linked 1-iminocyclitols) do not inhibit glycosidases (though much of this effect is probably due to the loss of the positive charge on the ring nitrogen atom).[74] Likewise, monoclonal antibodies against the G_{M3} lactam (lactam formed between the NeuAc carboxylate and the nitrogen atom of galactosamine) show no binding to G_{M3}, though they do bind the G_{M3} lactone.[75] This strong differentiation indicates that the lactam/lactone and uncyclized G_{M3} are likely in quite different conformations.

Better success has been achieved by replacing the glycosidic oxygen atom with carbon (C-glycosides) and sulfur (thioglycosides). Some examples are shown in Figures 18 and 19. Figure 18a shows PP-55b,[76] an excellent inhibitor of the mammalian Glc-P-Dol synthase, an enzyme that catalyzes the synthesis of a dolichyl phosphoglucose (Glc-P-Dol) from UDP-glucose and dolichyl phosphate. Glc-P-Dol is a precursor for the biosynthesis of the dolichol-linked oligosaccharide block which is transferred en masse to asparagine during N-linked glycoprotein synthesis. The inhibitor mimics both substrates, and two of the most notable features of this molecule are the use of uncharged mimics for the phosphate and pyrophosphate moieties of the substrates and the complete replacement of the sugar with a simple phenyl ring.

The compounds in 18b and 18c show C-glycoside and thioglycoside mimics of tri- and disaccharides. The lectin UEA-I from *Ulex europaeus* binds the H-antigen, a blood-group antigen which is also thought to be a receptor for some pathogens such as *H. pylori*.[8] The C-glycoside analogue of the blood-group antigen binds to the lectin with only slightly diminished affinity as compared to the normal saccharide, but is hydrolytically stable.[77] Galabiose (Galα1,4-Gal; Figure 18c) is a feature found in the Globo series of glycolipids and is bound by certain bacterial adhesins[78] such *as E. coli* PapG, and analogues are studied for preventing bacterial adhesion. The thioglycoside analogue was found to have a slightly altered conformation, and inhibited PapG–galabiose adhesion with a somewhat reduced though still quite respectable activity.[79]

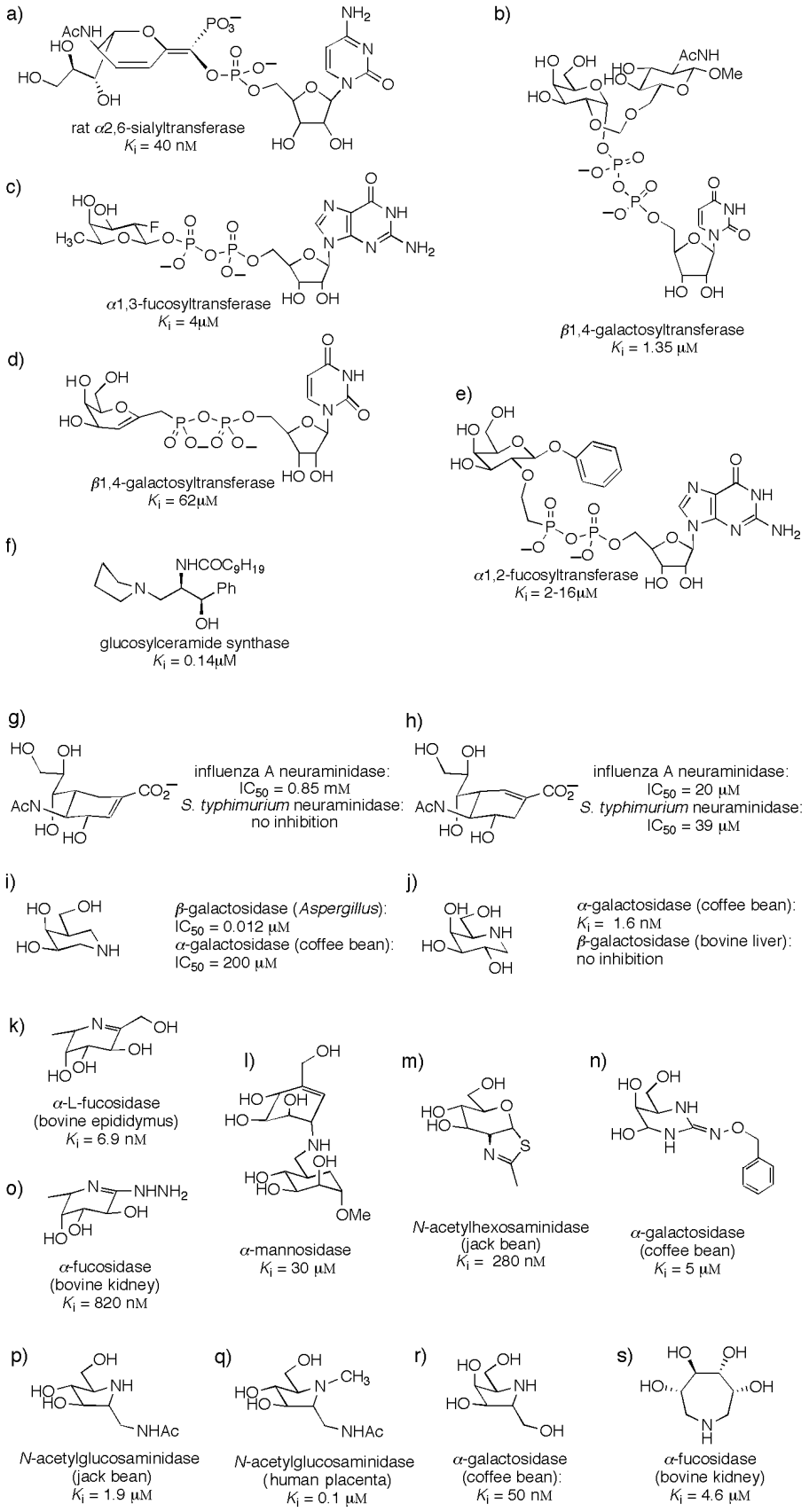


Figure 17. Selected glycosidase and glycosyltransferase inhibitors. a) Müller et al.;[162] b) Hashimoto et al.;[163] c) Murray et al.;[63] d) Schmidt and Frische;[164] e) Palcic et al.;[165] f) Miura et al.;[166] g), h) Vorwerk et al.;[167] i) Ichikawa et al.;[168] j) Bernotas et al.;[169] Look et al.;[170] k) Takayama et al.;[171] l) Cottaz et al.;[172] m) Knapp et al.;[173] n) Jeong et al.;[174] o) Schedler et al.;[175] p) Takaoka et al.;[176] q) Takebayashi et al.;[177] r) Wang et al.;[178] s) Moris-Varas et al.[179]

The G_{M3} ganglioside lactone (Figure 18d), in which the carboxylate of the sialic acid has formed an ester with the 2-OH group of the adjacent galactose, is a tumor-associated antigen;[80] the lactam, a more stable analogue, was strongly immunogenic and elicited antibodies that reacted with the lactone but not the ganglioside.[81]

Cholera toxin, the molecule responsible for the majority of the symptoms of infection by *Vibrio cholerae*, is an AB_5 hexameric toxin.[11] The five identical B subunits are responsible for binding to the cell surface ligand, the ganglioside G_{M1} (Figure 19a), and they do so in a coopoerative fashion.[18] The B subunits function to transfer and deliver the A subunit to the target cell, while the A subunit is a enzyme that catalyzes the ADP-ribosylation of G_S, a protein involved in signal transduction cascades. The modified G_S remains constantly turned "on" as a result, resulting in the overstimulation of adenylate cyclase.[11] The main binding determinants for cholera toxin within the G_{M1} ganglioside are the terminal sialic acid and galactose residues. An analogue of the ganglioside in which the reducing end glucose is replaced by a simple cyclohexanediol (Figure 19b) was found to have virtually identical binding and conformational properties as the natural ganglioside.[82]

5.1. Sialyl Lewis^x Binding to E-Selectin

A highly studied target for therapeutic intervention is the interaction of sialyl Lewis^x with the cell-surface receptor E-selectin. As discussed in the introduction, this binding event occurs early in the inflammatory response, and represents one of the initial steps in the recruitment of white blood cells to damaged tissue (Figure 20). Inappropriate recruitment or overrecruitment of leukocytes can have damaging effects. Many acute inflammatory conditions such as reperfusion injury and septic shock as well as chronic inflammatory diseases such as rheumatoid arthritis

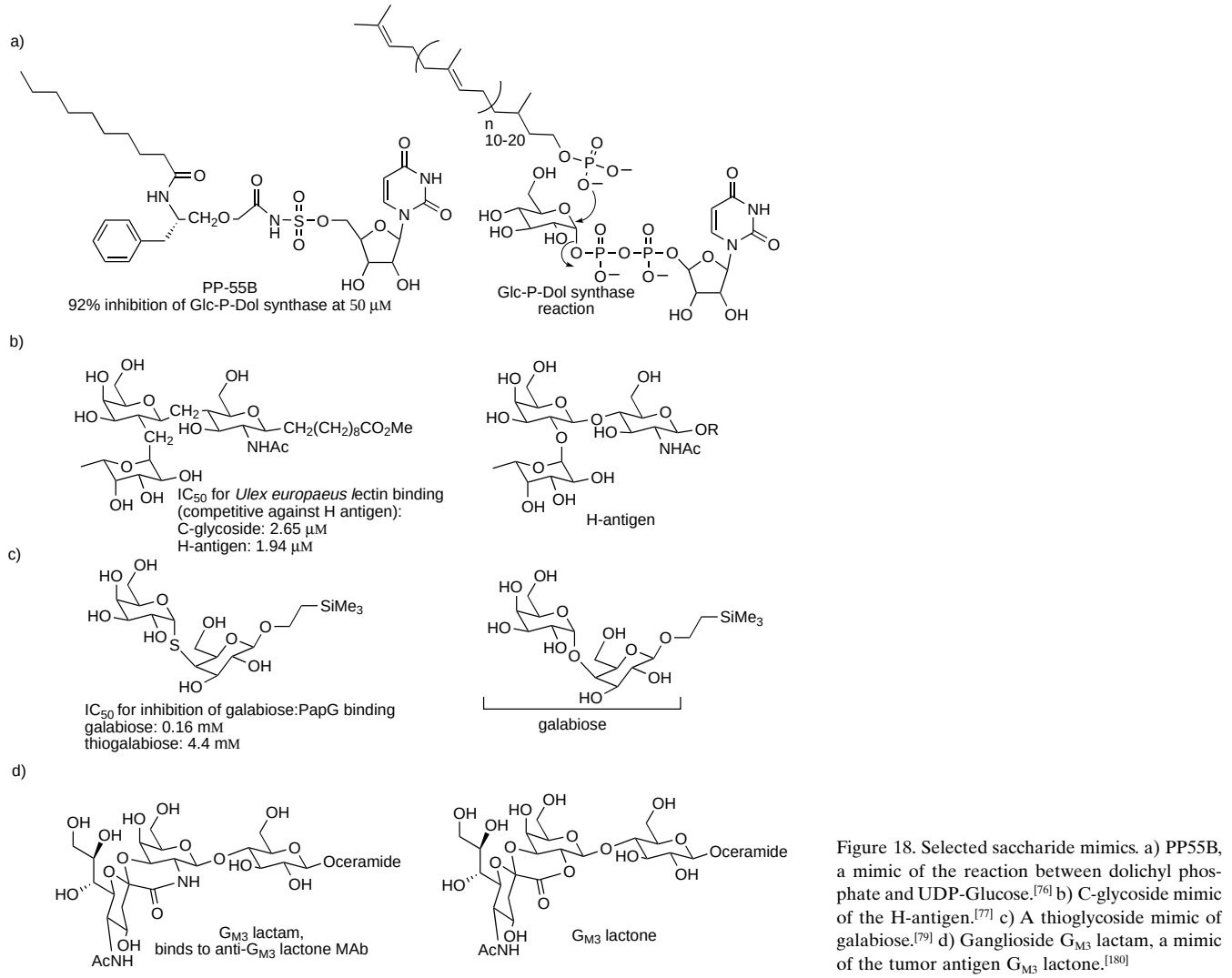


Figure 18. Selected saccharide mimics. a) PP55B, a mimic of the reaction between dolichyl phosphate and UDP-Glucose.[76] b) C-glycoside mimic of the H-antigen.[77] c) A thioglycoside mimic of galabiose.[79] d) Ganglioside G_{M3} lactam, a mimic of the tumor antigen G_{M3} lactone.[180]

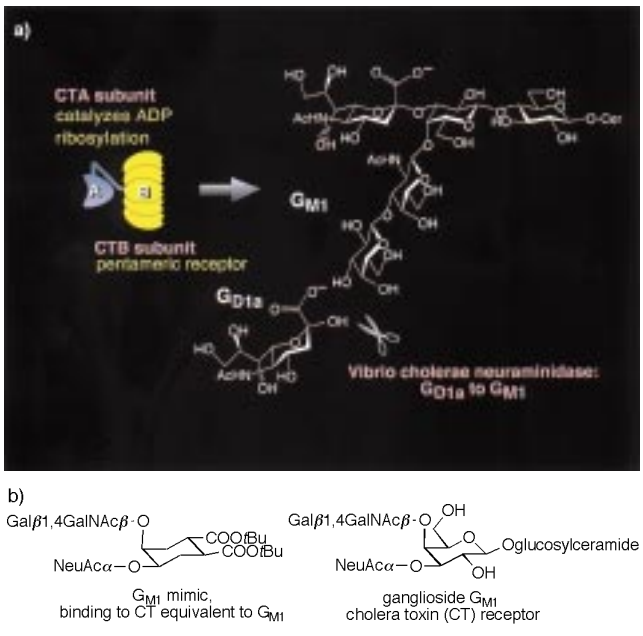


Figure 19. a) Binding of cholera toxin to cells. Vibrio cholerae neuraminidase removes sialic acid from ganglioside G_{D1a} to expose ganglioside G_{M1}, which is the receptor for cholera toxin. b) Inhibitor of cholera toxin binding to G_{M1}; a G_{M1} mimic built onto a cyclohexane scaffold.[82]

and asthma could be treated with antagonists of this interaction. In addition, since there is evidence that this interaction is responsible for the high metastatic potential of certain tumor lines such as melanomas (which are characterized by a high level of sialylated Lewis antigens),[55] intervention might provide a new target for cancer chemotherapy.

A large variety of sLe^X analogues have been designed and synthesized, based on 1) the known NMR structure of the tetrasaccharide bound to E- and P-selectins (which is noticeably different from that in solution, the main difference being in the conformation of the sialic acid residue; Figure 21);[83] 2) the crystal structure of the lectin and EGF-like domains of E-selectin;[34] and 3) knowledge of the important structural functional groups for selectin binding.[84, 85] A recent review[86] gives a condensed compilation of the field, some of which is highlighted here. The important interactions between sialyl $Lewis^X$ (or its sulfated derivatives) and E-, P-, and L- selectins are illustrated in Figure 21. For E-selectin recognition, all three hydroxy groups on fucose are necessary. This makes sense, as the 3- and 4-OH groups are believed to coordinate to a nearby calcium atom[34, 35] in a manner similar to the binding

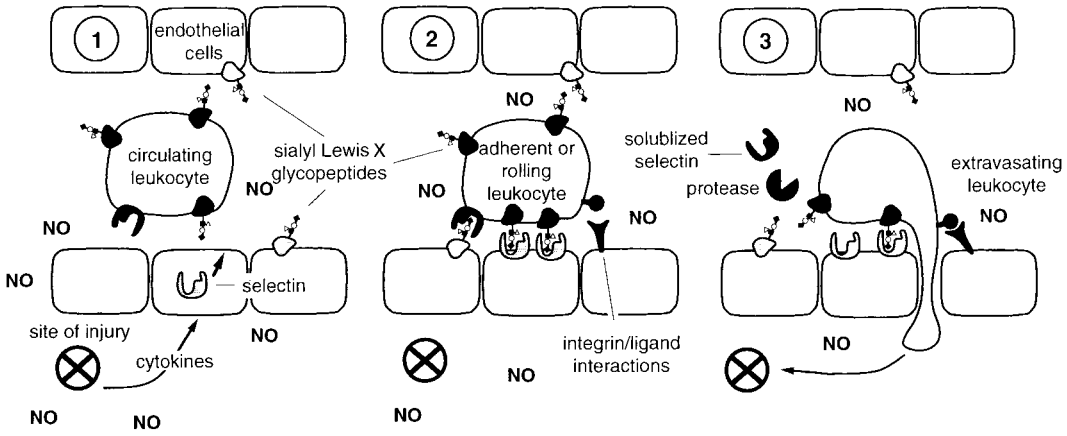
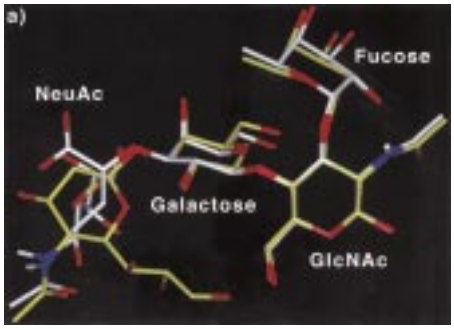


Figure 20. Schematic representation of the inflammatory reaction cascade. 1) Tissue injury occurs; cytokines and nitric oxide are released. 2) Selectins on cell surfaces interact with sialyl Lewis^X ligand. (E- and P-selectins are displayed on the endothelial cell surface, while L-selectin is on the leukocyte surface.) sLe^X is on the leukocyte surface or on the endothelial cell surface. sLe^X-6-sulfate is a preferred ligand for L-selectin. 3) Integrin-ICAM-1 interaction followed by extravasation of leukocyte.

of mannose by the mannose-binding protein (Figure 2). The fucose has, in fact, been replaced by D-mannose in a number of active compounds.[86] The 4- and 6-OH groups of galactose are also necessary, as is the carboxylate moiety of sialic acid.



GlcNAc appears to contribute no groups explicitly necessary for binding, and has been replaced with a variety of bifunctional linkers. Replacement of the sugars with aromatic or other hydrophobic groups (in hopes of improving the hydrophobic interactions) has resulted in compounds with higher affinity for E-selectin than sialyl Lewis^X (Table 1).

Another effective approach for creating higher affinity binders is to mimic the polyvalent nature of the leukocyte by incorporating sialyl Lewis^X into assemblies such as cross-linked liposomes or linear polymers (Figure 22). (For a thorough and recent review of polyvalency in biological systems, see Mammen et al.[15]) Similar approaches have been used to develop sLe^X and sulfo-sLe^X mimics to target the P- and L-selectins. Development of antagonists of P-selectin represents a significant challenge, as sLe^X binds to P-selectin

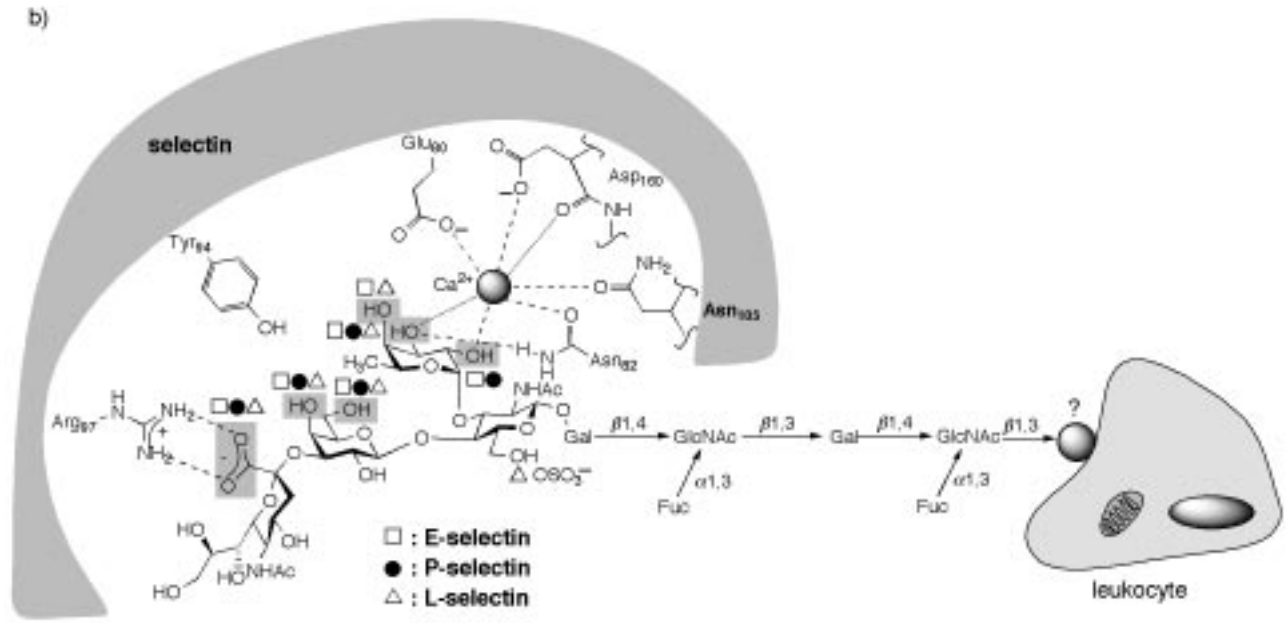
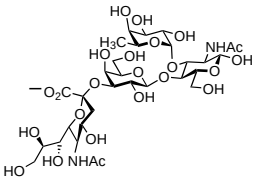
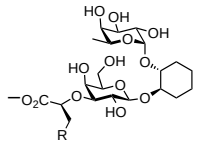
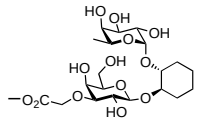
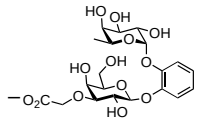
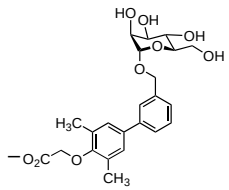
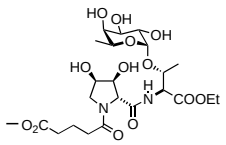
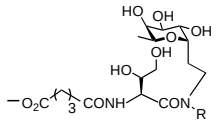
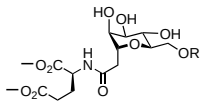
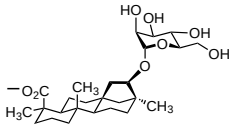
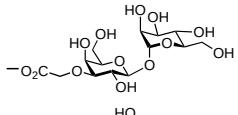
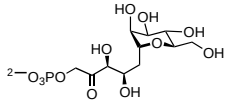
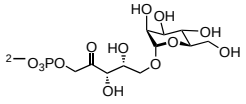


Figure 21. a) Solution conformations of sLe^X bound by L-selectin (white) and E- and P-selectin (yellow).[83] b) Binding determinants for sialyl Lewis^X binding to the selectins.

Table 1. Sialyl Lewis^X mimetics and their activities as compared to that of sLe^X.[83] n.d. = not determined.

		E-selectin	P-selectin	L-selectin
	(sLe^X)	0.7 mM	8 mM	4 mM
	R = cyclohexyl	80 μM[a]	–	–
	R = phenyl	350 μM[a]	–	–
		0.5 mM[b]	–	–
		> 10 mM[b]	–	–
		50 μM[c]	n.b.	n.b.
		50 μM[d]	n.b.	n.b.
	R = $CH_2CONH(CH_2)_{13}CH_3$	37 μM[e]	7 μM[e]	190 μM[e]
	R = H	0.3 mM[e]	4 mM[e]	> 5 mM[e]
	R = $(CH_2)_{15}CH_3$	53 μM[f]	2.2 μM[f]	7.6 μM[f]
	R = H	0.1 mM[f]	> 3 mM[f]	2.7 mM[f]
		> 3 mM[g]	80 mM[g]	> 3 mM[g]
		100 μM[h]	1.8 μM[h]	2.0 μM[h]
		800 μM[f]	5 μM[f]	40 μM[f]
		100 μM[f]	0.6 μM[f]	95 μM[f]

[a] Kolb and Ernst.[148] [b] Bamford et al.[149] [c] Dupre et al.[150] [d] Lin et al.[151] [e] Woltering et al.[152] [f] Wong et al.[153] [g] Ikeda et al.[154] [h] Hiruma et al.[155]

with a dissociation constant of about 8 mM. In addition, a secondary binding site for sulfotyrosine of the P-selectin glycoprotein ligand (PSGL-1) has been found to contribute significantly to binding (Figure 23). A short sulfated sLe^X glycopeptide[87] binds to P-selectin with $K_d \approx 70$ nM. So far, there is no good mimic reported to target the two binding sites of P-selectin, though the sulfated glycopeptide can, in principle, be prepared enzymatically on large scales as regeneration systems for PAPS and sugar nucleotides have been developed[86, 88] (Figure 23). L-Selectin binds a variety of sulfated saccharides, including heparin sulfate[89] and phosphomannan.[90] Recent evidence suggests sLe^X 6-sulfate may well be the natural ligand,[91, 92] and both 6-sulfo-sLe^X and the modified 6-sulfo-de-N-acetylated sLe^X-capped gangliosides have recently been prepared by total synthesis.[93] Interestingly, the de-N-acetylated version appears to be the better L-selectin ligand.

5.2. Heparin – Antithrombin

The "heparinoids," heparin and heparin sulfate, are heavily sulfated glycosaminoglycans consisting of repeating units of [D-glucosaminyl-α-1,4-hexuronosyl-β,1,4-], where the hexuronic acid is either glucuronate or iduronate (the C5 epimer of glucuronate).[94] The glucosamine is either N-sulfated or acetylated, and O-sulfation occurs at a variety of positions, particularly C2 of iduronic acid, C6 (and sometimes C3) of glucosamine, and occasionally C2 of glucuronate. Heparin is a more highly processed form of heparin sulfate, containing more iduronic acid and sulfate. Heparinoids show a broad range of biological effects including anticoagulant activity and enhancement of the stability and activity of acidic and basic fibroblast growth factors; they are in fact known to bind to well over a hundred proteins.[95]

Heparin's anticoagulant activity has been known and used clinically for over 60 years. Heparin inhibits the activity of both thrombin (factor IIa) and factor-Xa through interaction with antithrombin III (ATIII), as illustrated in Fig-

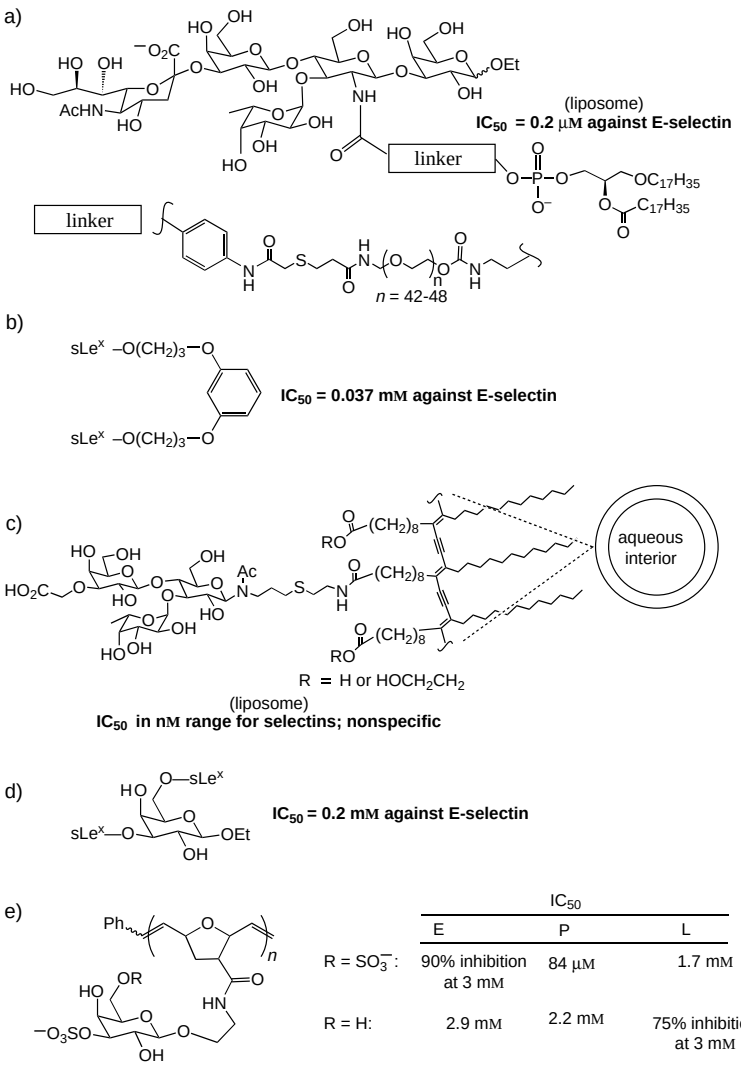


Figure 22. Polyvalent sLe^X mimetics. a) DeFrees et al.;[181] b) Miyauchi et al.;[182] c) Spevak et al.;[183] d) DeFrees et al.;[184] e) Manning et al.[146]

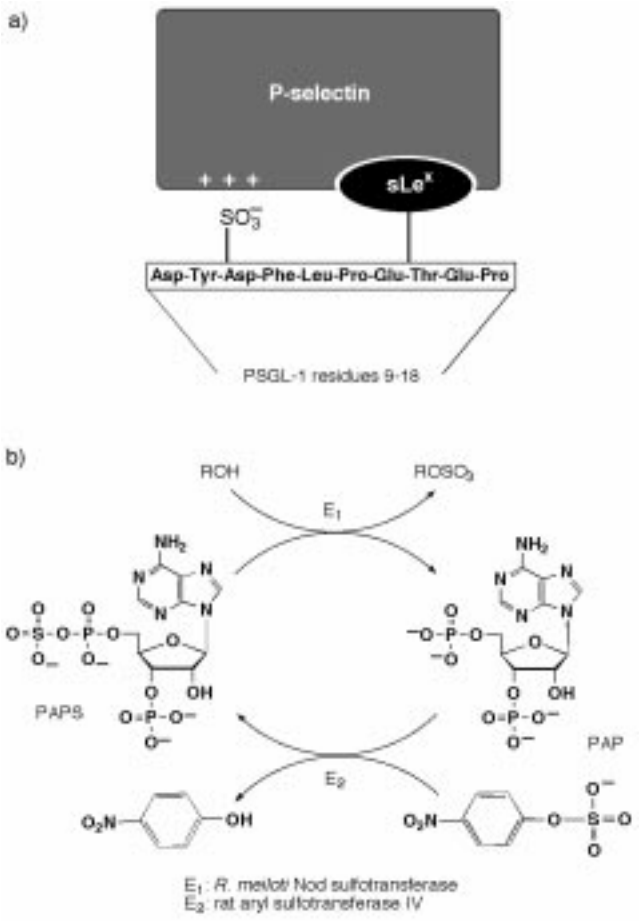


Figure 23. a) Additional interactions between P-selectin and the tyrosine sulfate of its glycoprotein ligand, PSGL-1.[87] b) A new regeneration of PAPS from PAP using *p*-nitrophenylsulfonate and a recombinant aryl sulfotransferase.[88]

ures 24 and 25. Binding of an ATIII recognition sequence (Figure 26a) causes a conformational change[96] in ATIII that is sufficient to allow its inhibition of factor Xa. Inhibition of thrombin, on the other hand, requires interaction of thrombin with both heparin-activated ATIII and directly with heparin itself. It is postulated that, in this case, heparin acts both as an activator of ATIII and an "adaptor" between it and thrombin.[97] Thus, a longer heparin molecule (ca. 18 saccharides long[97]) is required for inhibiting thrombin than is required for inhibiting factor Xa. Much of the binding interaction is electrostatic.

Grootenhuis and van Boeckel have constructed a model[98] of the association between the heparin pentasaccharide and ATIII. A three-dimensional structure of ATIII was postulated based on its homology to α1-antitrypsin, a protein of known structure. The necessity of residues 41 – 49 for heparin binding was previously known, and indeed these side chains fell within a region of concentrated positive charge. Docking of the heparin pentasaccharide into this region provided a model for the binding site in which the pentasaccharide is more or less bathed in lysine and arginine residues. The

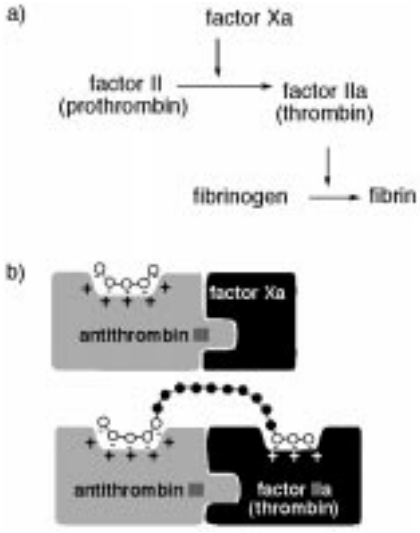


Figure 24. a) The last few steps of the coagulation cascade. b) Schematic representation of the interactions between heparin (represented by chains of spheres) and antithrombin III and between heparin and thrombin. Thrombin binding requires a larger heparin fragment (>18mer), whereas ATIII binding only requires a pentamer.

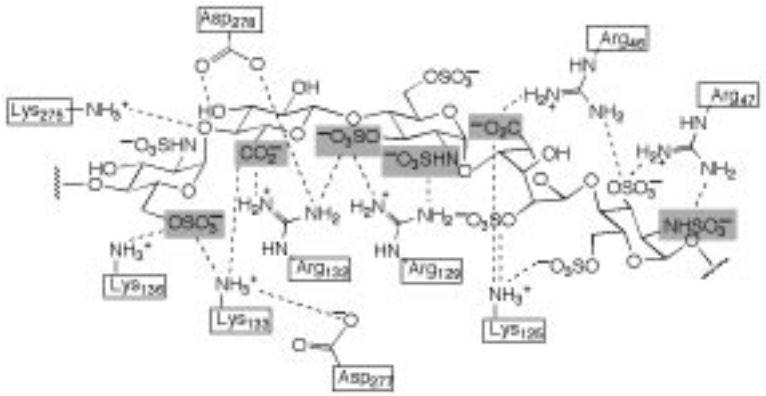


Figure 25. Important interactions between heparin and antithrombin III, based on a model by Grootenhuis and van Boeckel.[98]

critical functional groups (four sulfates and two carboxylates[94]) for binding to AT III are shown in Figure 26a. The N-sulfates can be replaced with O-sulfates, and many of the hydroxy groups can be methylated without loss of activity (in fact, with a slight gain in activity) against factor Xa.[94]

Conversion of the glucuronate to iduronate gives a moderate loss of activity, but this more than makes up for the ease of synthesis: A polymeric heparin mimetic can be constructed from dimeric units.[99] This has allowed the facile synthesis of the mimetic shown in Figure 26b, which is long enough to cause AT III inhibition of both factor Xa and factor IIa (thrombin) and, in fact, shows nearly the same thrombin inhibitory power as heparin. The

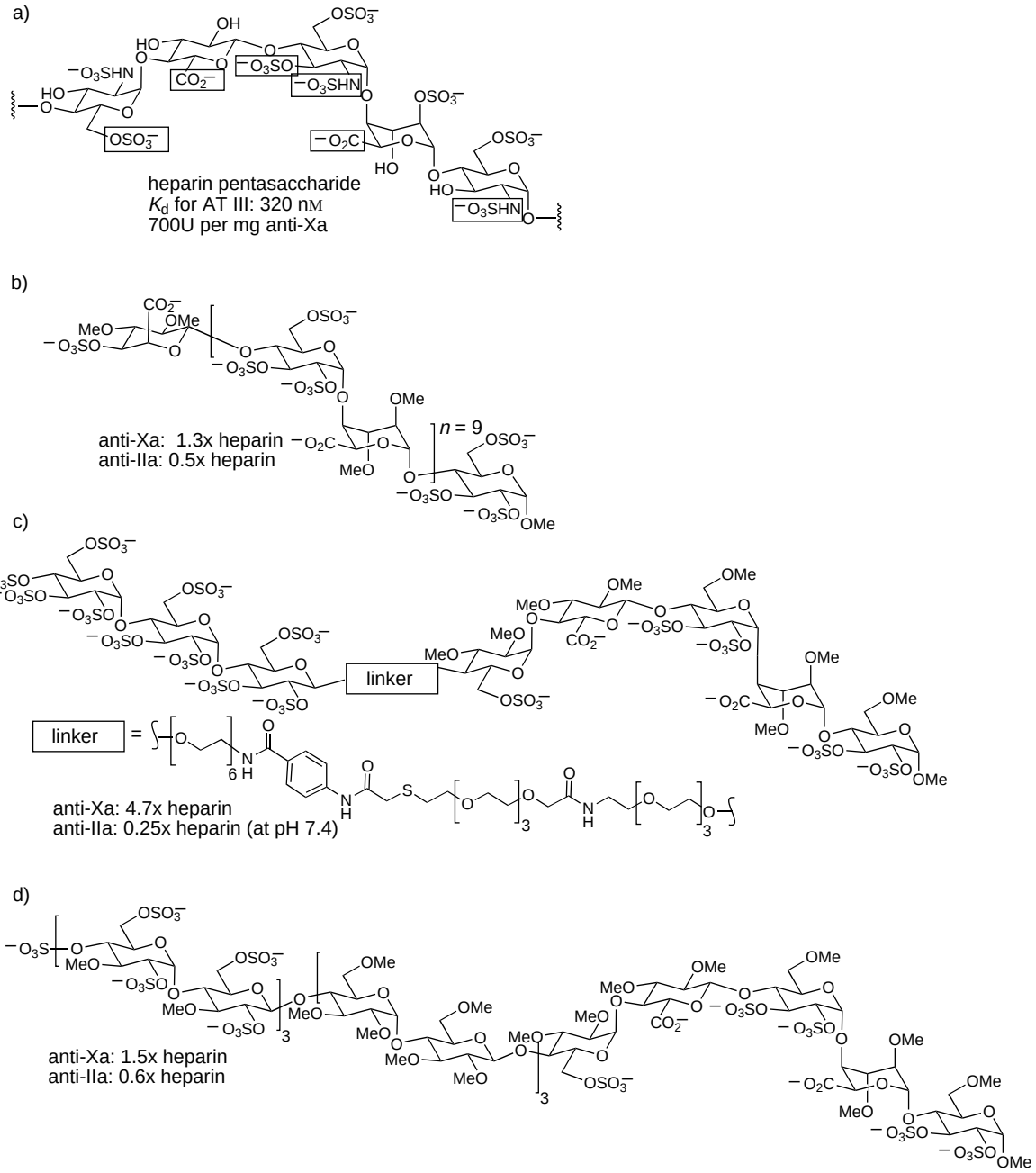


Figure 26. a) The recognition sequence for antithrombin III (AT III). b)–d) Heparin mimetics which bind both AT III and thrombin.

nonspecific binding of heparinoids to certain proteins such as platelet factor PF4 and basic proteins such as fibrinogen and the von Willebrand factor is thought to be responsible for the undesirable side effects of heparin therapy, (the most prominent of which are thrombocytopaenia and hemorrhages). Furthermore, it was postulated that reducing the charge density of the region between the AT III-binding region and the thrombin-binding region might reduce the nonspecific binding effects.[100]

Dreef-Tromp and colleagues[101] connected a thrombin-binding region to a AT III-binding region through uncharged flexible tethers. They attached a thrombin-binding region (fully sulfated maltotrioside) to the pentasaccharide shown in Figure 26a through either a flexible polyethylene-based linker (Figure 26c) or an uncharged polysaccharide linker. Both showed strong thrombin inhibitory power, though the saccharide linker was superior, perhaps due to the reduced flexibility. A similar compound with a different thrombin-binding domain is shown in Figure 26d. Preliminary data regarding PF4 binding and bleeding time in the presence of the latter heparin mimetic suggests that it may display fewer side effects than heparin.[100]

It is worth noting that the binding site of heparin for fibroblast growth factor (FGF) is quite distinct from the AT III-binding site. The consensus sequence for FGF binding is a 14mer called oligo-H: GlcA-β1,4-GlcNSO$_3$-α1,4-[IdoA(2S)-α1,4-GlcNSO$_3$]$_5$-α1,4-IdoA-α1,4-GlcNAc.[102] Randomly derivatized dextrans (derivativized with carboxylmethyl, benzylamide, and benzylamidesulfonate) can substitute weakly for heparin[103] with regard to FGF activation and stabilization, but have minimal anticoagulant activity.

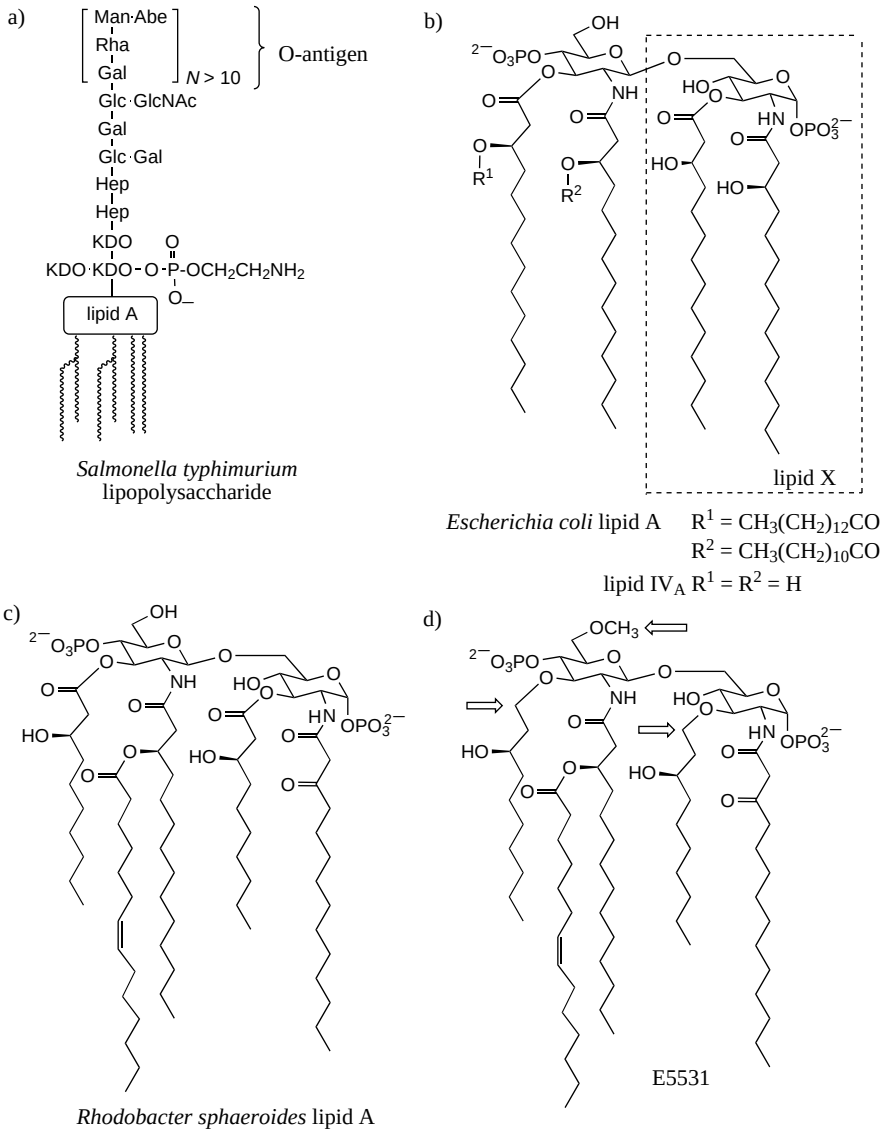


Figure 27. Gram-negative bacterial lipopolysaccharides. a) *Salmonella typhimurium* LPS. b) Lipid A from several bacterial species: *E. coli* lipid X, lipid A and lipid IV$_A$. c) *Rhodobacter sphaeroides* lipid A. d) E5531. Abe = abequose, Hep = L-glycero-D-mannoheptose, KDO = 2-keto-3-deoxyheptanoic acid.

5.3. Lipid A, LPS Binding Protein, and CD14

Gram-negative bacteria are characterized by a second membrane external to the peptidoglycan matrix. A major constituent of this membrane is lipopolysaccharide (LPS). Although lipopolysaccharides differ between bacterial species, they follow the same general format. The "anchor" is a large structure called lipid A (Figure 27), a phosphorylated disaccharide with multiple fatty acid tails attached through ester and amide linkages. This is elaborated with a "core" polysaccharide and finally capped with a repeating structure called the O-antigen, a highly variable and species-specific saccharide. Lipopolysaccharides from many bacterial species are highly toxic to humans (hence their common name "endotoxin"), and are responsible for septic shock.[104]

Septic shock is a serious problem, and an unfortunate example of the old expression "the treatment was a success but the patient died". Antibiotic therapy can kill pathogenic gram-negative bacteria, but the endotoxin released by the lysed bacteria may cause more acute and lethal symptoms than the original infection. Toxicity occurs through a process beginning with the binding of LPS to a soluble binding protein (LBP) in serum. This complex binds to the glycosyl phosphatidyl inositol (GPI) linked protein CD14 and the toll-like receptor TLR2 on the surface of macrophages and neutrophils, resulting in the production of a variety of cytokines by the cells (Figure 28).[105, 106] The hope of finding an agent to

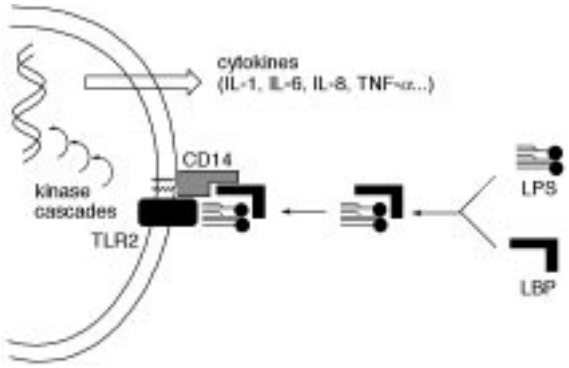


Figure 28. Schematic representation of the immune activation by bacterial LPS.[105, 106]

combat endotoxemia has received a boost with the discovery of glycolipids that are both nontoxic and have endotoxin antagonistic activities, such as lipid A from *Rhodobacter sphaeroides*[107] and the lipid A precursors lipid X and lipid IV_A (Figure 27).[104] The *Rhodobacter* lipid A is unfortunately unstable in aqueous solution, being hydrolyzed at the ester positions. Synthesis of a stabilized mimetic, E5531 (6′-O-methylated and with ester links replaced by ethers),[104, 108] has provided a potential therapeutic agent to combat septic shock. This compound shows potent LPS-antagonistic activity and is currently in clinical trials. A variety of other compounds which mimic either the *Rhodobacter* lipid A or LPS-antagonistic lipid A precursors lipid X and lipid IV_A are also under study, and these compounds were recently reviewed by Chaby.[105]

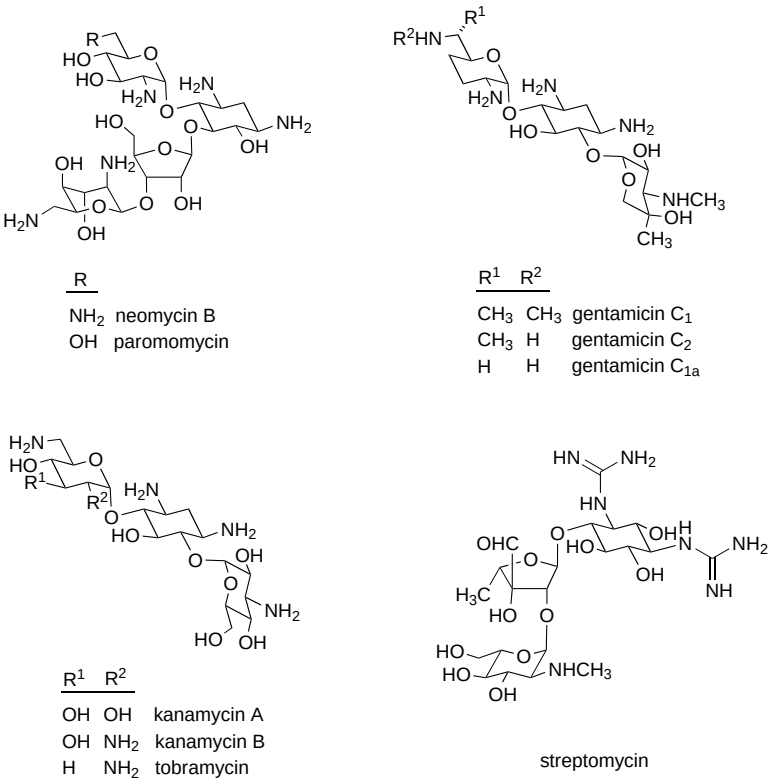


Figure 29. Structures of selected antibiotics.

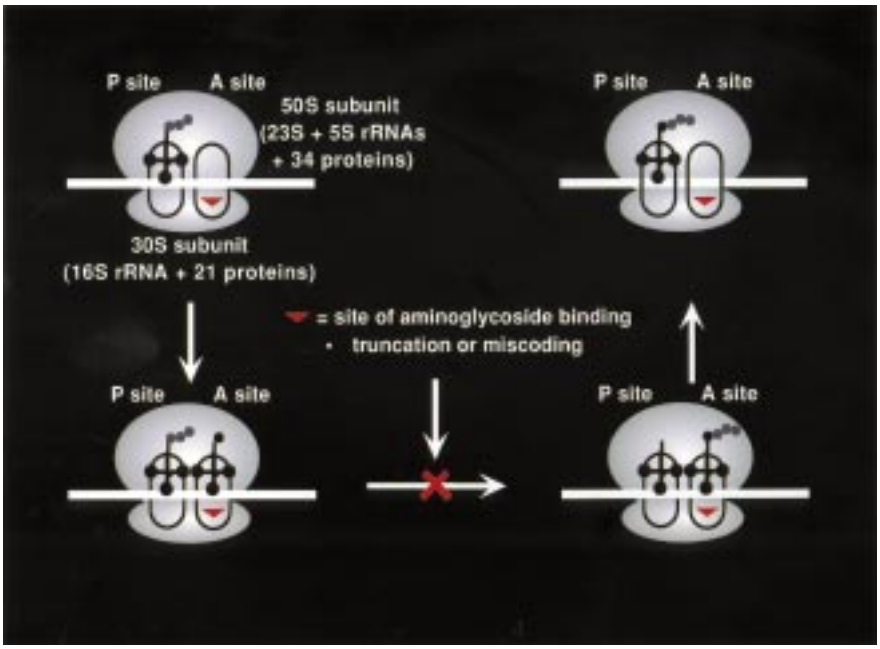


Figure 30. Schematic representation of aminoglycoside interactions with the bacterial ribosome.

5.4. Aminoglycoside – RNA Interaction

Many aminoglycoside antibiotics such as neomycin and streptomycin (Figure 29) interact with the 16S subunit of the bacterial ribosome, inhibiting translation and causing miscoding (Figure 30).[13] The aminoglycosides have several disadvantages. Many of the aminoglycosides are orally inactive and/or toxic to humans, and must be administered topically or by injection. They generally concentrate in the kidneys, and many show strong nephrotoxicity.[109] Additionally, there are many aminoglycoside resistant bacterial strains that overcome antibiotic challenge through a variety of mechanisms, including mutation within the ribosomal binding site (Figure 31a) and covalent modification (acetylation, phosphorylation, or adenylylation; Figure 31b) of the antibiotic.[110, 111] Mimetics of aminoglycosides that are less toxic, more stable, and that are not substrates for modification enzymes could be therapeutically useful.

An interesting structural feature of these antibiotics is the presence of *trans*-1,3-hydroxyamine or *cis*-1,3-diamine motifs. A recent study indicates that the 1,3-hydroxyamine group can interact strongly with both the phosphodiester backbone and the Hoogsteen face of guanine[42] (Figure 5). This realization has led to the design and synthesis of a variety of aminoglycoside mimetics that conserve this motif (Figure 32a). The affinities of these compounds for the neomycin binding region

of the bacterial 16S RNA was evaluated by surface plasmon resonance[112] (Figure 32b), and many of the compounds proved to be good binders. Some of these have displayed excellent antibiotic activity.[113] Computer modeling suggests that these analogues may bind in a manner similar to that of the natural products (Figure 33). Other groups have also been actively involved in the discovery of aminoglycoside mimetics. Many of the structures designed contain extra amino, guanidino, or 1,3-hydroxyamine functionalities.[114, 115] Although many of these compounds have not yet been tested against resistant strains, they may be poor substrates for aminoglycoside-modifying enzymes.

5.5. Sialic Acid Interactions with Haemagglutinin and Neuraminidase

Influenza virus has two proteins that bind sialic acid on the surface of the host cell. One, the sialoside binding haemagglutinin, is responsible for the initial binding of the influenza virus to the host cell. The other, neuraminidase (sialidase), cleaves sialic acids and is important for allowing the young virions to escape and infect new cells. Both interactions are potential targets for intervention.[116, 117] Haemagglutinin is a homotrimeric molecule found in many copies on the surface of the virus. Important interactions between haemagglutinin and the sialoside receptor are shown in Figure 34. Since the individual haemagglutinin binding sites show weak affinity for

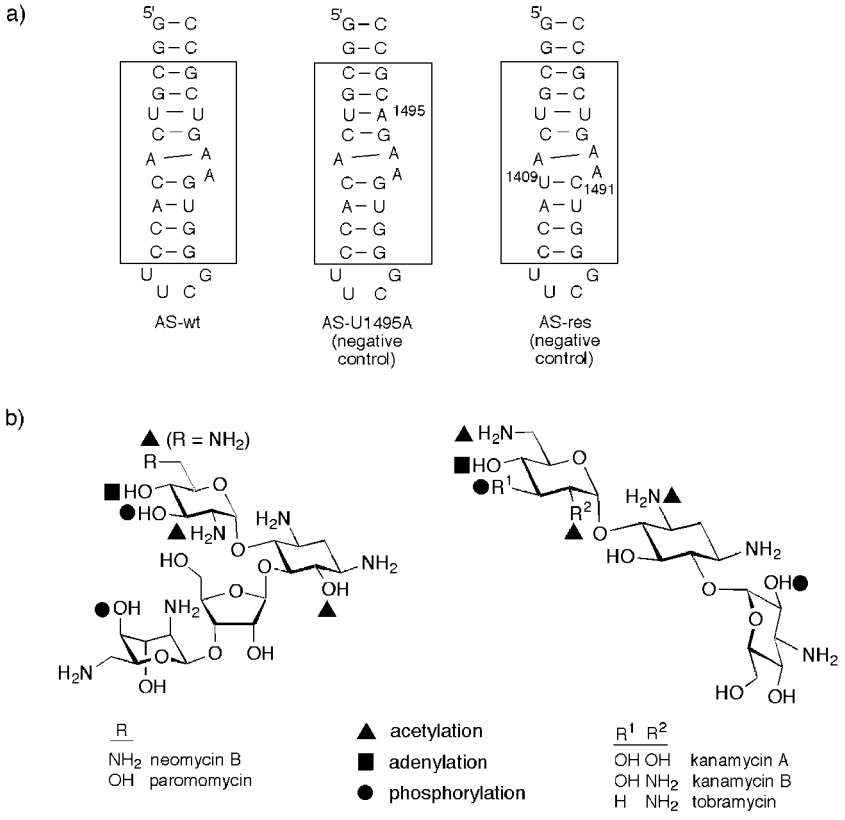


Figure 31. Modes of bacterial resistance to neomycin and related antibiotics. a) Normal binding site of neomycin, a small section of the 16S ribosomal subunit; the homologous 16S RNA section in a neomycin-resistant mutant; and a control RNA with the binding determinants for neomycin removed. b) Neomycin deactivation by covalent modification.

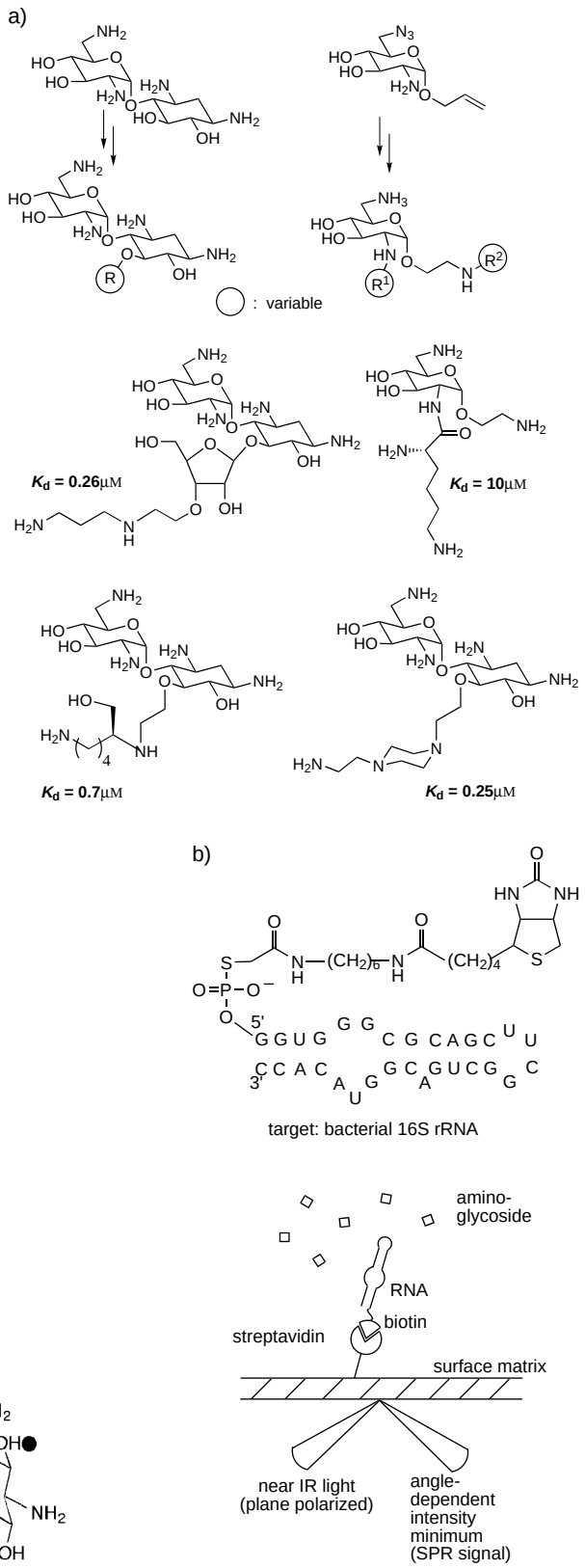


Figure 32. a) A strategy for aminoglycoside mimetic synthesis.[113, 185] b) Surface plasmon resonance (SPR) assay for evaluating binding to 16S rRNA.[112] (In SPR, the angle at which a minimum is observed in the reflected light intensity is sensitive to the refractive indices on either side of the support, a thin gold film. The refractive index of the (solution) side will be affected by binding events.)

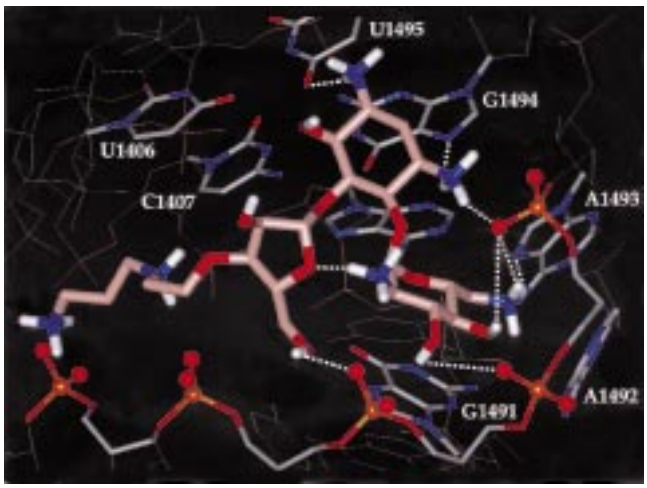


Figure 33. Model of neomycin mimetic binding to the same site as the natural aminoglycoside paromomycin, based on modeling studies using the NMR structure of a section of the ribosomal 16S RNA.[43]

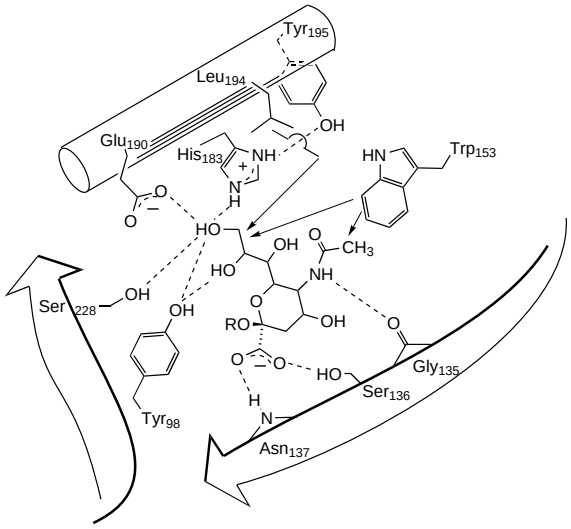


Figure 34. Important interactions between viral haemagglutinin and the receptor, sialic acid. Dashed lines represent potential hydrogen bonds, whereas solid arrows represent hydrophobic interactions (e.g. between the haemagglutinin side chain Trp153 and the sialoside C8 and C9). The drawing is based on the crystal structure published by Weis et al.[186]

monovalent sialosides ($K_d = 2-3$ mM),[118–120] most monomeric sialic acid analogues have shown poor efficacy in preventing viral infection, although one fluorescent derivative displays micromolar inhibition of hemagglutination.[121] This compound has several extra hydrophobic groups which may interact with hydrophobic binding groups in the binding site. Another approach to improve affinity is to mimic the polyvalent nature of the cell surface. To this end, a variety of structures such as liposomes and polymers with pendant sialosides or sialic acid mimics (especially C-linked sialic acid) have been prepared (Figure 35). The compound in Fig-

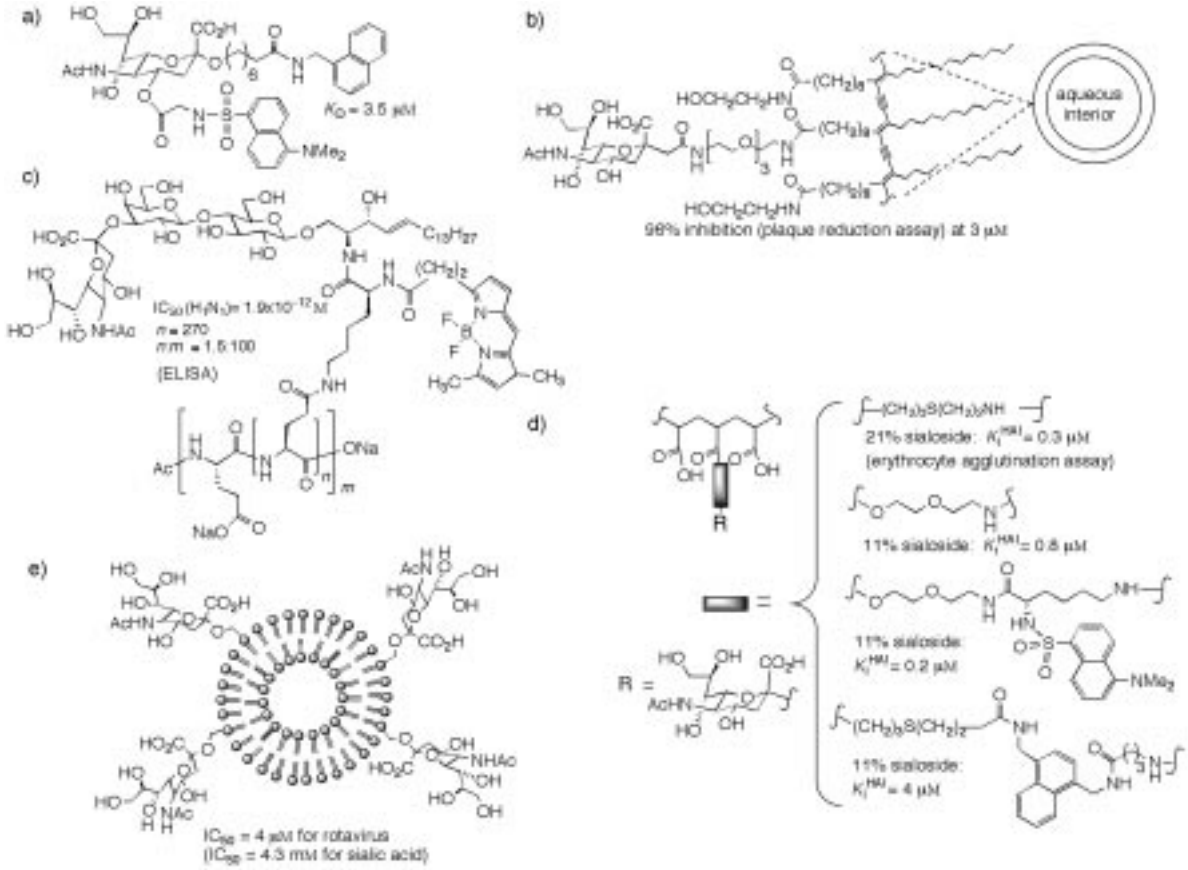


Figure 35. Inhibitors of haemagglutinin-mediated viral binding. For reference, the sialic acid monomer (methyl glycoside) has an IC_{50} of 2 mM.[118] a) A fluorescent, tightly binding monomeric sialoside.[121] b) Cross-linked liposome of Spevak and co-workers.[187] c) Polyglutamate with pendant sialyllactose from Kamitakahara et al.[188] d) Polyacrylamide-based inhibitors from Choi et al.[189] Sialosyl phospholipid-based liposomes, which show efficacy against rotovirus, may be applicable to influenza virus as well.[190]

ure 35 d, a picomolar inhibitor of haemagglutinin binding, contains a hydrophobic group which contributes additional binding affinity through interaction with the hydrophobic groups of the receptor. In addition, it has a possible advantage in that it does not simply have sialic acid, but a sialyllactose moiety. Upon neuraminidase cleavage of the terminal sialic acid, galactose residues will be exposed that may allow rapid viral clearance by receptor-mediated endocytosis (e.g. by the asialoglycoprotein receptor;[122] Figure 36).

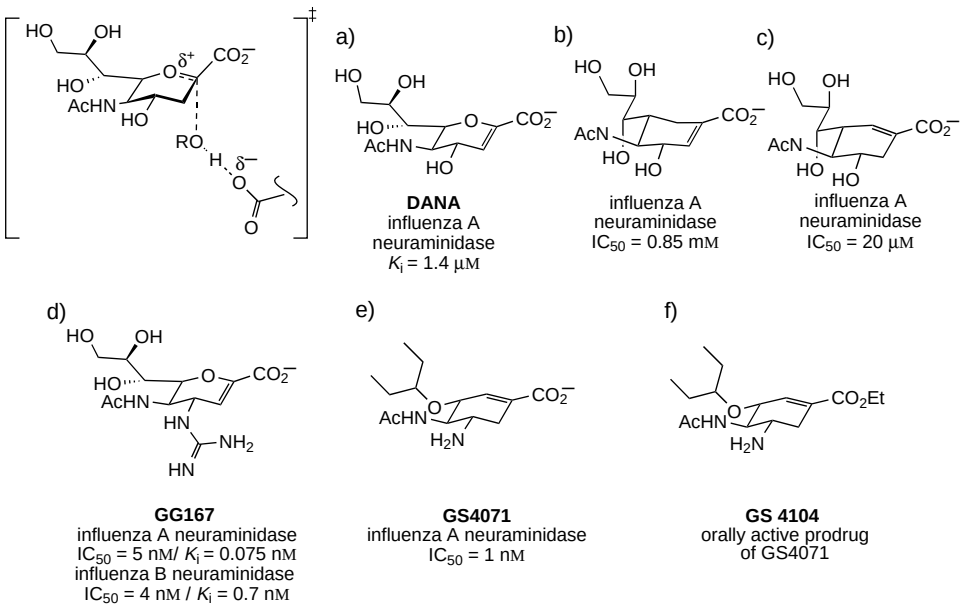


Figure 37. Inhibitors of influenza neuraminidase. a) DANA. b), c) carbocyclic DANA analogues.[167] d) GG167.[126] e), f) GS4071 and the orally active prodrug GS4104.[191]

Unlike haemagglutinin, neuraminidase strongly binds the hydrolytic transition state, rather than the ground state, of sialosides. The dehydrated form of neuraminic acid, 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid (DANA, Neu5Ac2en; Figure 37 a), has been known for many years to be a moderately good inhibitor of the enzyme.[123] Attempts to mimic the transition state received several great boosts. First, the crystal structure of neuraminidase was solved in 1983.[124] Several years later, isotope effect studies on the mechanism of neuraminidase action, which by analogy with other glycosidases might be expected to proceed through an S_N1-type mechanism, supported this view of the catalytic mechanism.[64]

The crystal structures[41, 125] of the enzyme in the presence of added NeuAc and DANA provided better information regarding the recognition of substrate (Figure 38) by the enzyme, and showed that NeuAc bound to the active site in a distorted conformation, supporting the proposed mechanism. Using this information, a large number of transition state analogues have been rationally designed[126, 127] and synthesized (Figure 37). A number of these have shown excellent inhibition of a variety of influenza neuraminidases, and some, such as GS4104 and GG167, are in clinical trials. (GS4104 is an esterified prodrug of GS4071, which can be absorbed by the digestive tract. The ester is then cleaved by natural esterases.) The discovery of a hydrophobic binding pocket flanked by Ala 246 and Ile 222 that is unused by sialic acid but used by GS4071 has allowed the design of inhibitors that take advantage of contacts not used by the natural substrate,[128] though none of these proved better than GS4071 (administered by nasal spray).

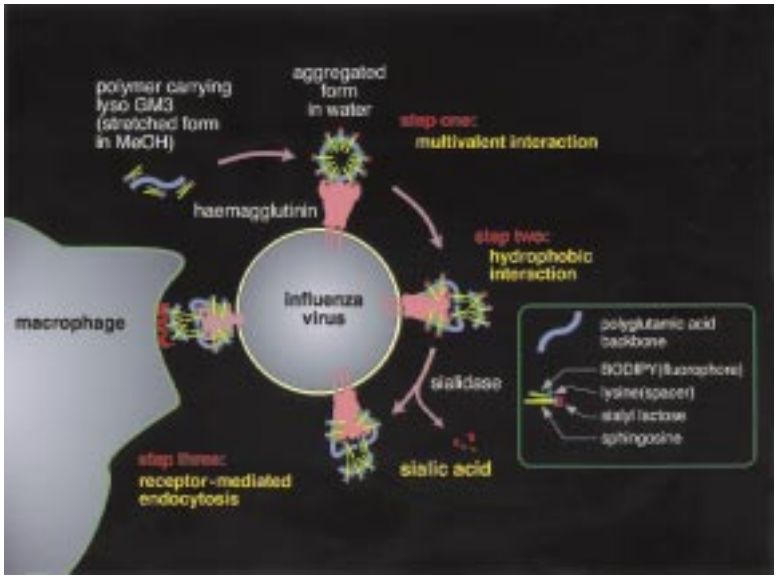


Figure 36. Schematic representation of the binding, desialylation, and proposed receptor-mediated endocytosis of influenza virus by the polyvalent compound in Figure 35 c.

5.6. Glycoproteins, Neoglycoproteins, and Glycoprotein Mimics

Many proteins are glycosylated, particularly in eukaryotic cells. The glycans are typically linked either to the side-chain amide nitrogen atom of asparagine (N-linked) or to hydroxylated amino acids such as serine and threonine. Other linkages are known, including surprising C-linkages to tryptophan,[1] but these are much less common. Protein glycosylation can have a variety of functions.[1, 3] The sugars can substantially affect the physical properties of the protein in solution. This is most familiar in the case of the mucins, a class of heavily O-glycosylated proteins responsible for the sliminess of mucous.[9] In addition, the saccharide can affect the stability, function, and targeting/clearance properties of the glycoprotein. Glycoprotein/glycopeptide mimics are created for a variety of reasons, including investigation of the

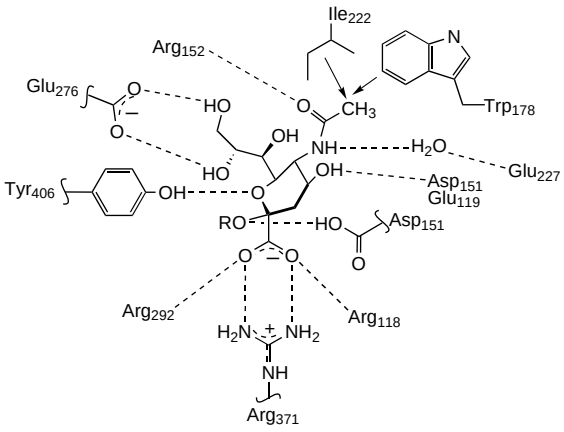


Figure 38. Important hydrogen-bonding, electrostatic, and hydrophobic interactions between neuraminidase and the substrate. Dashed lines represent hydrogen bonds and electrostatic interactions, whereas solid arrows represent hydrophobic interactions (e.g. between the methyl group of the AcNH group and the side chains of Ile 222 and Trp 178). The drawing is based on the crystal structure published by Varghese et al.[41]

saccharide function, modification of the targeting properties, improvement of the biological activity, and ease of synthesis. Different strategies used for preparation of glycoprotein mimics are shown in Figure 39. In the first approach, the protein is unchanged, but the saccharide is replaced. In a second scheme, the protein is unchanged, the saccharide may or may not be changed, but the linkage between saccharide and protein and the location of glycosylation are altered. In the most radical approach, the protein scaffold is either altered or completely abandoned.

The first approach requires synthetic techniques for the preparation of homogeneous glycoproteins, which are unfortunately difficult to make. Glycoproteins obtained by fermentation techniques are very heterogeneous,[1] but chemical synthesis of glycoproteins can be difficult. Solid-phase peptide synthesis (SPPS) with glycosylamino acids frequently suffers from relatively low yields, particularly as the length of the protein and the saccharide are increased. The presence of glycans, particularly O-glycans, precludes the use of many of the protecting groups commonly used for SPPS since the glycosidic linkages are unstable under the deprotection conditions. Nevertheless, progress is being made in synthetic techniques.[129–132] Synthetic procedures have been worked out for preparing glycosylamino acid building blocks suitable for SPPS.[133, 134] Chemical synthesis has been used, for example, to create "mucin-like" peptides for use as cancer vaccines.[135] Enzymatic approaches also show promise. Two enzymatic approaches toward

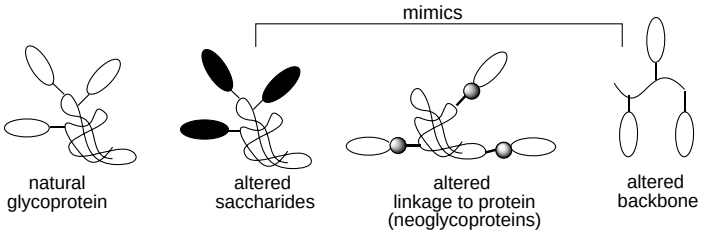


Figure 39. Approaches for designing glycoprotein mimics.

the production of homogenous N-linked glycoproteins from a heterogeneous population produced in vivo are shown in Figure 40. In the first, an endoglycosidase is used to digest all but the desirable sugar residue(s) from the protein (or alternatively proteases can be used to condense glycopeptide fragments); glycosyltransferases are then used to reelaborate the saccharide.[136] The second approach involves the direct endoglycosidase-catalyzed transfer of glycans from a oligosaccharylasparagine donor directly to the glycoprotein.[137, 138] Another synthetic approach is based on intein-mediated protein splicing.[139, 140] Recent studies in this lab indicate that glycoproteins can be prepared by an intein-promoted reaction of a extein–intein fusion protein with a glycopeptide containing an N-terminal cysteine (Figure 41).

An alternate approach to the construction of glycoproteins mimics is the preparation of a "neoglycoprotein," in which the

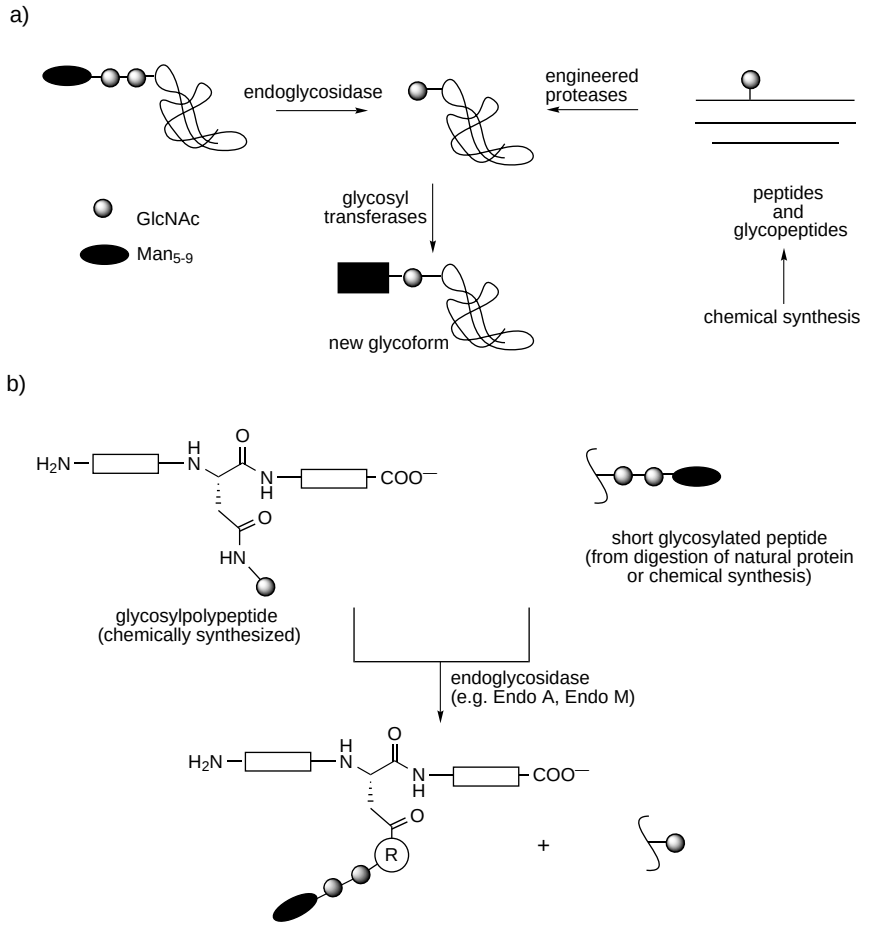


Figure 40. Enzymatic techniques for synthesizing homogeneous glycoproteins (see text for details).

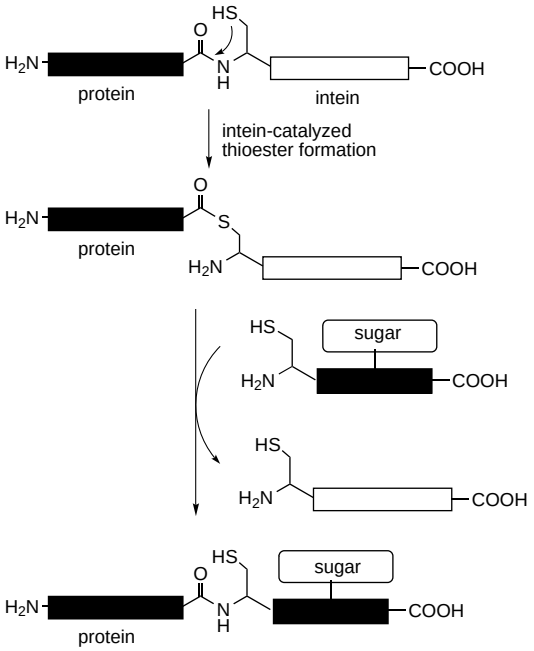


Figure 41. Use of inteins to condense biologically produced proteins with synthetic glycopeptides.

saccharide is attached through an unnatural linkage.[141] Connection of the saccharide to the peptide may be accomplished by chemical or enzymatic techniques, and may be site-specific or random. For example, Wang and co-workers[138] used the approach shown in Figure 40b to prepare a C-linked glycopeptide which was a competitive inhibitor of glycoamidases, enzymes that cleave the entire saccharide from N-linked proteins (by hydrolysis of a side-chain amide functionality). Bertozzi and co-workers have developed a strategy for the chemoenzymatic assembly of O-linked glycopeptides by enzymatic oxidation of the 6-OH group of a GalNAc peptide to the aldehyde followed by oxime formation with a hydroxyaminoglycoside.[142] Alternatively, the construction of several saccharide vaccines has required the chemical attachment of sugars or saccharide conjugates to a carrier protein such as keyhole limpet hemocyanin.[135]

The last approach, the construction of glycoprotein mimics in which the protein backbone is either heavily modified or replaced with a non-peptidic scaffold, is useful for mimicking proteins in which the polypeptide serves little function except as a framework for multiple copies of the glycan. The mucins, for example, have been mimicked by clustered O-glycosides linked to unnatural peptide or non-peptidic backbones.[143, 144] Sialyl Lewis^X and other saccharides have been linked to polyacrylamide,[145] "ROMP" polymers (oligomers prepared by ring-opening metathesis polymerization),[146] and dendritic superstructures.[147]

6. Summary and Outlook

The examples described in this review give a sampling of some of the recent progress in the field of carbohydrate mimetic design. A number of carbohydrates has successfully been mimicked with simpler, more synthetically accessible, and more stable molecules. The preparation of carbohydrate mimics has also allowed the inclusion of new functional groups that can increase the affinity of the molecule for its target. The principles used in the design of the mimics described will hopefully prove to be widely applicable, and allow for the design of therapeutics that intervene in biologically important carbohydrate recognitions.

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